Hospital Library
JUL 27 1940

PHYSIOLOGICAL REVIEWS

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

	PAGE
CARBON MONOXIDE ANOXEMIA. *Esther M. Killick*	313
FLUCTUATIONS IN BODY IODINE. *William T. Salter*	345
THE RÔLE OF POTASSIUM IN PHYSIOLOGICAL PROCESSES. *W. O. Fenn*	377
THE PORPHYRINS IN HEALTH AND DISEASE. *Konrad Dobriner and C. P. Rhoads*	416

VOL. 20, NO. 3

Issued July, 1940

BALTIMORE, U. S. A.

Entered as second-class matter, January 15, 1921, at the Post Office at Baltimore, Md., under the act of March 8, 1879

Made in United States of America

HISTORY OF THE AMERICAN PHYSIOLOGICAL SOCIETY

To commemorate the Semicentennial of its foundation the American Physiological Society has published an illustrated history, with index, of its development since 1887. The book contains articles by William H. Howell and Charles W. Greene, with a foreword by Walter J. Meek, and was distributed in May, 1938.

The book of about 220 pages is in case-binding and costs $2.50.

Orders should be sent to

D. R. HOOKER, *Managing Editor*, 19 W. Chase Street, Baltimore, Md.

PHYSIOLOGICAL REVIEWS

PHYSIOLOGICAL REVIEWS is controlled by the Board of Publication Trustees of the American Physiological Society. The Trustees appoint the Editorial Board.

Board of Publication Trustees. W. J. MEEK, WALLACE O. FENN and H. C. BAZETT.

Editorial Board. J. H. BURN, Oxford, England; A. J. CARLSON, Chairman, Chicago; E. M. K. GEILING, Chicago; R. W. GERARD, Chicago; E. W. GOODPASTURE, Nashville; A. BAIRD HASTINGS, Boston; LAURENCE IRVING, Swarthmore; P. E. SMITH, New York, and D. R. HOOKER, *Managing Editor*, Baltimore.

The Editorial Board selects the subjects and authors of all articles which are published, the aim being to provide concise but comprehensive reviews of the recent literature and present status of various subjects in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology. Each volume will consist of from sixteen to twenty articles, making a total of about five hundred pages per volume. The numbers will be issued quarterly in January, April, July and October of each year.

Subscriptions and **orders** will be received in advance only. The subscription price, net postpaid, is $6.00 in the United States, $6.25 in Canada and $6.50 elsewhere. Single issues may be purchased at $2.50 each. Communications should be sent to:

LAURA E. CAMPEN, *Secretary of Publications*
19 W. CHASE ST., BALTIMORE, MD.

PHYSIOLOGICAL REVIEWS

VOL. 20 JULY, 1940 No. 3

CARBON MONOXIDE ANOXEMIA

ESTHER M. KILLICK

Department of Industrial Physiology, London School of Hygiene and Tropical Medicine, London, England

Carbon monoxide derives its physiological interest from its property of combining with hemoglobin in a manner apparently identical with oxygen. The hemoglobin, however, has a very much greater affinity for carbon monoxide than for oxygen, and therefore carbon monoxide is a potent cause of anoxemia when it is mixed with the inspired air.

The combination of carbon monoxide with reduced hemoglobin, in the absence of oxygen, and the dissociation of carboxyhemoglobin, follow the same laws as govern the combination of oxygen with reduced hemoglobin and the dissociation of oxyhemoglobin. The curve relating the partial pressure of the gas to the percentage saturation of the hemoglobin is identical in shape for both gases, and its position is influenced by the same factors in both cases, i.e., pH, temperature, and salt content (Douglas, Haldane and Haldane, 1912). The difference lies in the range of partial pressures; while the hemoglobin of human blood becomes half saturated with oxygen at a partial pressure of about 30 mm., it is half saturated with carbon monoxide, under the same conditions of temperature, pH, etc., when exposed to a partial pressure of about 0.125 mm.

When blood is exposed to a mixture of O_2 and CO the Hb is divided between the two gases, the reaction obeying the law of mass action; i.e., the proportion of Hb combined with either gas depends upon the relative partial pressures of the O_2 and CO, and upon a constant which expresses the relative affinity of the blood for the two gases (Haldane and Smith, 1897)

$$\frac{[HbCO]}{[HbO_2]} = \frac{K[CO]}{[O_2]}$$

On the basis of this fact partition curves may be constructed for any blood for which the equilibrium constant (K) is known; these curves

relate the partial pressure of CO to the per cent saturation of the Hb with CO when the partial pressure of O_2 is kept constant. Similar curves can be constructed for O_2 when the partial pressure of CO is constant. Such curves are rectangular hyperbolas, provided the sum of the partial pressures of the two gases is sufficient to saturate the Hb completely. If this condition does not obtain, the curves show a curious "hump" in the area where the Hb is not completely saturated (Douglas, Haldane and Haldane, 1912). The rectangular hyperbola, that is, expresses only the reaction

$$HbO_2 + CO \rightleftharpoons HbCO + O_2$$

and when reduced Hb is present also the relationship is altered. But if the reduced Hb be ignored the hemoglobin that is combined still follows the same law and is divided between the oxygen and carbon monoxide according to the relative partial pressures of the two gases. The existence of this "hump" in the partition curves when reduced hemoglobin is present means that in the presence of low partial pressures of oxygen and carbon monoxide the addition of carbon monoxide will actually increase the proportion of oxyhemoglobin as well as that of carboxyhemoglobin. Haldane and Smith (1897) actually observed this effect; mice subjected to low oxygen tension seemed to improve when the carbon monoxide in the atmosphere was increased. At the time the accuracy of the observation was doubted, but it was realized later that it was a demonstration of the nature of the partition curve at these low partial pressures.

The value of the equilibrium constant (K) is practically independent of pH, salt content, laking, or purification of the hemoglobin (Haldane et al., 1912; Hartridge, 1912b; Roughton, 1934). Factors such as CO_2 tension, therefore, which markedly affect the oxygen or carbon monoxide dissociation curves of the blood, have no effect on the partition of hemoglobin between oxygen and carbon monoxide. This partition, however, is greatly affected by light, and also by temperature. The effect of light was observed by Haldane and Smith (1897) and has since been confirmed by numerous workers; Hartridge (1912) demonstrated that the most active part of the spectrum in this respect was the ultraviolet end, and concluded that it hastened the dissociation of HbCO in the presence of oxygen. The value of K varies inversely with the temperature at which equilibration occurs; Hartridge (1912) found a fall in the saturation of the hemoglobin with carbon monoxide of $\frac{1}{2}$ per cent for each 1°C. rise in temperature; Hartridge and Roughton (1923) found a temperature coefficient of 1.3 per 10°C.

Hartridge and Roughton in their work on the kinetics of hemoglobin have shown that a consideration of the velocity of the various reactions involved in the combination of hemoglobin with oxygen and carbon monoxide provides the explanation of many of the facts previously demonstrated experimentally (Hartridge and Roughton, 1923; Roughton, 1934). Light has been shown to accelerate the velocity of dissociation of HbCO. The great affinity of carbon monoxide for hemoglobin is due to the fact that HbCO dissociates very much more slowly than HbO_2, although oxygen combines with hemoglobin about 10 times as fast as does carbon monoxide under similar conditions of pH, temperature, and concentration of reagents.

The equilibrium constant (K) also varies considerably between animals of different species. Krogh (1909–1910) showed that the bloods of the rabbit and the ox differed markedly in their affinity for CO; his figures indicate a value of K = 288 for ox blood (37°C.) while for the bloods of different rabbits K varied from 115 to 205. The temperatures at which the different bloods were equilibrated varied, however, and when corrected to 37°C. the values of K for rabbit blood are approximately 83–140. Douglas and Haldane (1911) showed that the bloods of animals of different species exhibited marked differences in percentage saturation with carbon monoxide when equilibrated with the same air-carbon monoxide mixture, and similar differences were found between individuals of the same species. Burrell, Seibert and Robertson (1914) assessed the susceptibility of a number of small animals and birds to carbon monoxide by exposing the animals to atmospheres containing low concentrations of carbon monoxide and observing their symptoms. Barcroft and his co-workers (1924–25) determined the value of the equilibrium constant for the bloods of a number of vertebrate species, and found that at 15°C. it varied from K = 570 for a dog to K = 120 for a rabbit. They found considerable individual variation in mice, horses, and rabbits, but much less variation between human individuals. The value of K was correlated with certain spectroscopic properties of the blood; the distance, in Angström units, between the position of the α band of oxyhemoglobin and that of carboxyhemoglobin, defined as the "span," was shown to bear a definite relationship to the value of K in all the bloods examined. The curve relating these two properties was represented by the equation:

$$\log K = 0.05 \text{ A.U.}$$

where A.U. was the "span" in Angström units. The existence of individual differences among mice was confirmed by Killick (1937) who

found values for K, determined at 37°C., ranging from 118 to 278. She also found individual differences in humans, and records values of K (at 37°C.) for 4 individuals of 233, 240, 246 and 272 (1936); the first three values lie within the limits of experimental error, but the fourth value, which was obtained repeatedly for that individual, indicates a difference outside these limits. Douglas, Haldane and Haldane (1912) record values for two individuals of 246 and 299 (at 37°C.). Sendroy, Liu and Van Slyke (1929), using a gasometric method to estimate the percentage saturation of the hemoglobin, found an average value for K of 210 in six individuals; the variation between these individuals was within the limits of experimental error. There seems little doubt that differences do exist between the hemoglobins of human individuals in this respect, but until more data are available it is impossible to say how wide the variation is. In most of these experiments the constant was determined by equilibration of whole blood with a series of air and carbon monoxide mixtures, in some diluted blood was used. The percentage saturation of the blood with carbon monoxide after equilibration was determined either by a gasometric method (Van Slyke and Robscheit-Robbins, 1927), by means of the reversion spectroscope (Hartridge, 1912a), or by the carmine method (Douglas and Haldane, 1912).

Effects of breathing carbon monoxide. The relationship between the partial pressures of oxygen and carbon monoxide and the saturation of the blood with carbon monoxide enables one to predict approximately the final blood saturation of a man who is breathing air containing a small amount of carbon monoxide, but it gives no information as to the speed with which that saturation will be reached. The rate at which the percentage saturation increases in different animals is proportional to the respiratory exchange per unit of body weight (Haldane, 1895b), and therefore in small animals absorption will be rapid and the final equilibrium will be reached much more rapidly than in man; the mouse reaches equilibrium with the mixture it is breathing twenty times as rapidly as man, and hence its use as an indicator of atmospheres dangerous to man (Haldane, 1895b).

In the human being the rate at which the body takes up carbon monoxide depends mainly on two factors, the partial pressure of carbon monoxide in the air breathed, and the volume of pulmonary ventilation; it also depends on the rate of blood flow through the lungs, but under normal conditions the blood flow and the pulmonary ventilation usually vary together. It is important to know how fast the percent-

age saturation of the blood will rise when an individual is exposed to a given concentration of carbon monoxide in air, in order to predict the nature of the symptoms and to decide the limits of safety. When a subject at rest inhales a mixture of air and carbon monoxide the saturation of his blood with carbon monoxide increases in a regular manner. Haldane (1935a) has described this process for the saturation of the tissues with nitrogen when man is subjected to increased atmospheric pressure, and the saturation of hemoglobin with carbon monoxide proceeds in a similar way in the living body (Killick, 1936). Figure 1[1] shows the form of the curve relating the percentage saturation of the blood to the duration of exposure; this curve may be applied to any

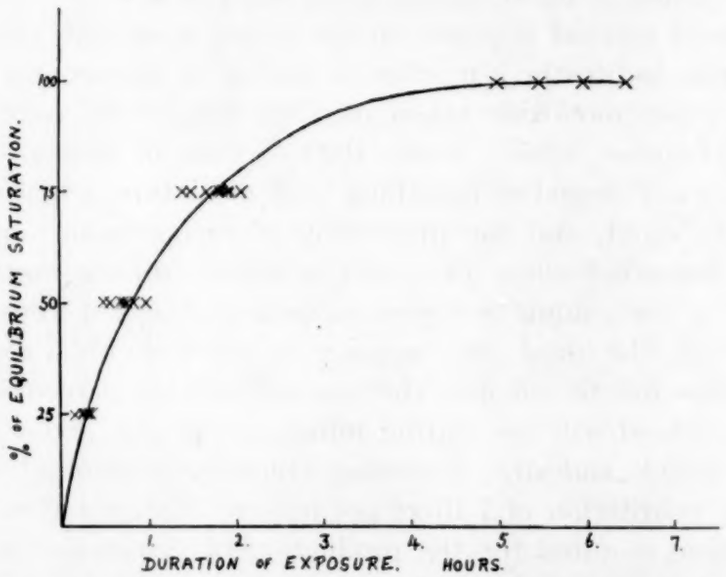

Fig. 1. Rate of increase of blood saturation (as percentage of equilibrium value) during exposure of the human subject to carbon monoxide.

concentration of carbon monoxide in air, since the saturation of the blood is expressed as a percentage of the final equilibrium saturation. It will be seen that half the final saturation is reached in about 47 minutes; these figures apply to very low concentrations of carbon monoxide, and Henderson, Haggard et al. (1921a) found that whereas half the final saturation was reached within one hour when the atmospheric concentration of carbon monoxide did not exceed 0.07 per cent, above this concentration the percentage saturation of the blood seemed to rise somewhat more rapidly. If one knows approximately

[1] The data embodied in this diagram were obtained from the experiments reported in 1936, but have not previously been recorded in this form.

what the equilibrium constant for the blood is, it is easy to calculate what will be the final percentage saturation with carbon monoxide for any given partial pressure of the gas, and so one can deduce the rate at which carbon monoxide will be absorbed. It is also possible to predict approximately how long any given concentration of carbon monoxide can be breathed before symptoms of poisoning appear, a point of obvious practical importance. It must be remembered that when an individual is doing muscular work the process of absorption is more rapid; according to Henderson and his co-workers (Henderson, Haggard, Teague, Prince and Wunderlich, 1921a) absorption is twice as rapid during moderate exercise (walking) as it is while sitting at rest, and three times as rapid during fairly hard work.

The second method depends on the observation that when an individual begins to breathe a mixture of carbon monoxide and air, about half the carbon monoxide taken into the lungs with each breath is retained (Haldane, 1895b; Veale, 1922). This, of course, only holds during the early period of breathing such a mixture, while absorption is relatively rapid, and the proportion of carbon monoxide retained increases somewhat when breathing is deeper during exercise. The knowledge of the volume of carbon monoxide absorbed, thus obtained, together with the total gas capacity of the individual's circulating blood, enables one to calculate the rate at which the percentage saturation of the blood will rise during inhalation of any given mixture of carbon monoxide and air. Assuming a blood volume of 5 liters, and a pulmonary ventilation of 7 liters per minute, Haldane (1895b) states that the time required for the production of symptoms in an adult man depends on the time required for the inhalation of about 660 cc. carbon monoxide, or the absorption of about 330 cc.

It is important to remember that the reaction

$$HbO_2 + CO \rightleftharpoons HbCO + O_2$$

is a reversible one, and therefore the carbon monoxide gradually disappears from the circulating blood when a poisoned man or animal breathes fresh air. The rate of elimination of carbon monoxide is governed by the same factors as is its absorption. When an individual whose blood is, for example, 30 per cent saturated with carbon monoxide, is brought into fresh air, carbon monoxide is eliminated and the percentage saturation falls fairly rapidly at first (to 20 per cent in less than two hours (Sayers, Yant, Levy and Fulton, 1929)), but the process slows down as the percentage saturation decreases, and it is

usually many hours before the last traces of carbon monoxide disappear. Sayers and his co-workers found about 5 per cent saturation 7 to 8 hours after the end of the exposure. The elimination of carbon monoxide is hastened by any factor that increases the volume of pulmonary ventilation and the circulation rate of the blood, or that raises the partial pressure of oxygen in the alveoli (Stadie and Martin, 1925–26).

It has been claimed that ultra-violet light hastens the elimination of carbon monoxide from the blood; Koza (1929) poisoned rabbits with illuminating gas to the stage of unconsciousness, and found that in the irradiated animals the percentage of carboxyhemoglobin was reduced by $\frac{3}{4}$ in a period during which the controls lost only $\frac{1}{4}$ of their carboxyhemoglobin. He suggests that the effect is due to dissociation of carboxyhemoglobin in the skin capillaries under the influence of the radiation. This explanation is rather difficult to accept unless one assumes that the carbon monoxide is eliminated through the skin, and it seems possible that the difference between the radiated animals and the controls was due to greater activity of the former, a factor that is very liable to falsify such experiments (Haggard and Greenberg, 1933).

Mode of action of carbon monoxide. The percentage saturation of the blood with carbon monoxide represents fairly accurately the degree of anoxemia to which the individual is subjected, because carbon monoxide acts by preventing the normal carriage of oxygen in the blood, and not as a direct tissue poison. It is known that carbon monoxide can act as a tissue poison by inhibiting the enzymic oxidative systems in the tissues, but this effect is only evident at relatively high partial pressures of the gas. Warburg (1926) showed that the respiration of yeast is inhibited in a mixture of carbon monoxide and oxygen, the degree of inhibition depending on the ratio of carbon monoxide to oxygen, but inhibition was not evident until the partial pressure of the carbon monoxide was nearly 5 times as high as that of the oxygen; De Meio, Kissin and Barron (1934) compared the metabolism of isolated tissues in mixtures of oxygen with carbon monoxide and with nitrogen, and found that certain tissues did show inhibition with carbon monoxide. This effect was demonstrated in the living body by J. B. S. Haldane (1927); he repeated J. S. Haldane's experiment (1895a) by exposing rats to a total pressure of two atmospheres of oxygen and one atmosphere of carbon monoxide. The blood of the animals was 98.3 per cent saturated with carbon monoxide under these conditions, but they remained almost unaffected because the blood carried an amount

of oxygen in simple solution that was sufficient for the needs of the body at rest. When, however, another atmosphere of carbon monoxide was added severe symptoms were evinced, and Haldane concluded that the carbon monoxide, at this high partial pressure, affected some substance in the tissues. It is clear from the first part of this experiment that the inhibitory effect on the tissues is of no significance under ordinary conditions in the living animal, because the deprivation of oxygen by the combination of carbon monoxide with hemoglobin would be fatal long before the partial pressure of carbon monoxide was high enough to affect tissue oxidative systems. Carbon monoxide acts, then, as a physiologically inert gas, except for its power of combining with hemoglobin, and this statement is confirmed by the indifference to carbon monoxide of animals and tissues without hemoglobin, e.g., cockroaches (J. S. Haldane, 1895a) cultures of chick neuroblastic tissue (Haggard, 1922). Carbon monoxide does not appear to be oxidized in the living body (J. S. Haldane and Smith, 1896; J. S. Haldane, 1900; M. Krogh, 1915), although Fenn and Cobb (1932a, b) have shown that it is oxidized by certain isolated tissues when they are placed in an atmosphere of 80 per cent carbon monoxide and 20 per cent oxygen. In addition to combining with the hemoglobin of the blood carbon monoxide combines with the muscle hemoglobin, but the affinity of the muscle hemoglobin for carbon monoxide is much lower than that of the blood hemoglobin. Theorell (1934) has shown that myoglobin is similar to hemoglobin in its combination with oxygen and with carbon monoxide, but the equilibrium constant that determines its partition between the two gases is only 13.8 for horse myoglobin at 37°C. (20.9 at 15°C.); Theorell notes that this value for K fits exactly on Barcroft's curve relating log K to "span," (see p. 315). When carbon monoxide is inhaled only a small proportion of the absorbed gas combines with the myoglobin; Gscheidlen (1869) stated that in estimating blood volume by the carbon monoxide method muscle hemoglobin took up about $\frac{1}{20}$ of the carbon monoxide absorbed. This is consistent with Whipple's observation (1926a) that in dogs the myoglobin is about 15 per cent of the total hemoglobin, taking into consideration the relatively low affinity of the myoglobin for carbon monoxide. There is no direct evidence as to the effects produced in the living body by a partial saturation of the myoglobin with carbon monoxide, but Millikan (1936) has demonstrated that myoglobin fulfills an important function as a short period oxygen store during muscular contraction, and Whipple (1926b) found that the quantity of hemoglobin in the skeletal muscles

was dependent on the degree of muscular activity. It is interesting that muscular weakness is a prominent symptom of acute carbon monoxide poisoning (see p. 339), although there is no evidence of any connection between this symptom and the combination of carbon monoxide with the muscle hemoglobin.

Barkan (1938) has described a pigment which he calls pseudohemoglobin, and which is an intermediate stage between hemoglobin and bile pigment. It accompanies hemoglobin to the extent of 5 per cent, and its relative affinity for carbon monoxide is significantly higher than that of hemoglobin. Barkan claims that the properties of this pigment are useful in diagnosing carbon monoxide poisoning, and may prove useful in elucidating some of the problems of chronic poisoning. The presence of this pigment may account for the recorded persistence of carbon monoxide in the blood for periods of days after acute poisoning.

The symptoms of carbon monoxide anoxemia in man. The symptoms of acute carbon monoxide poisoning in man have often been described (Glaister and Logan, 1914a; Sayers and Davenport, 1930; Haldane and Priestley, 1935b; Drinker 1938a), and may be considered in relation to the degree of saturation of the blood with carbon monoxide. Table 1 indicates the sequence of symptoms.

As indicated in table 1 the symptoms are accentuated by muscular exercise, and serious symptoms appear at a lower percentage saturation of the blood when the individual is working than when he is quiet. The symptoms vary also with the rate at which the percentage saturation of the blood is increasing. A man who is exposed to a high concentration of carbon monoxide, as for example after a colliery explosion, may experience very few symptoms until muscular weakness supervenes, and he is near unconsciousness. During exposure to low concentrations of carbon monoxide the symptoms appear gradually and their severity varies with the duration of exposure. If the percentage saturation of the blood with carbon monoxide is raised fairly rapidly, say to 35 per cent, and then maintained at that level for a short period (10–20 min.) the symptoms and after effects will be slight. If, instead, the carbon monoxide is gradually absorbed, so that the 35 per cent saturation represents equilibrium and the whole process occupies 6 or 7 hours, the result will be a severe headache. After an exposure of this type the symptoms are usually most severe during the period of recovery, and the headache that is characteristic of carbon monoxide anoxemia often does not begin until after the period of exposure to the gas.

It is interesting that when an individual is taken up to a high altitude by railway, there is usually a latent period of several hours before the symptoms of mountain sickness appear (Haldane and Priestley, 1935c). The relation of the symptoms and after effects of inhalation of carbon monoxide to the duration of exposure as well as to the atmospheric

TABLE 1

PER CENT SATURATION OF BLOOD WITH CO.	SYMPTOMS
0–10	Nil
10–20	Nil at rest; during exercise tightness across forehead, slight headache
20–30	Frontal headache may occur at rest; exertion causes dizziness, palpitation, and hyperpnea
30–40	At rest, headache, frontal or occipital, increased pulse rate, deeper breathing, palpitations, nausea; exertion causes dizziness, dimness of vision, abnormal increase of pulse rate and respiration, sometimes collapse
40–50	All symptoms more marked; vision, hearing, and intelligence begin to be impaired; muscular weakness on attempted exertion, with greater likelihood of collapse. Nausea and vomiting
50–60	Syncope; increased respiration and pulse rate; coma
60–70	Coma with depressed heart action and respiration; possibly death
70–80	Weak pulse and respiration; respiratory failure and death

The data in this table are taken from the sources mentioned above, and from Sayers, Yant, Levy and Fulton (1929) and Killick (1936).

concentration has been quantitatively expressed by Henderson, Haggard, et al. (1921b) as follows:

When duration of exposure in hours × concentration in parts per 10,000
= 3; effect is nil
= 6; effect just perceptible
= 9; headache and nausea
= 15; dangerous

This quantitative statement provides a very useful guide in assessing quickly the probable effects of any given exposure, but of course it does not allow for the fact that very low concentrations of carbon monoxide would never produce symptoms because the percentage saturation of the blood at equilibrium would be too low.

The symptoms of carbon monoxide poisoning resemble closely the symptoms of anoxemia due to altitude, or to a low partial pressure of oxygen produced in any other way, but there is one marked difference. In carbon monoxide poisoning syncope, or a feeling of faintness, is much more prominent than hyperpnea, whereas the opposite may be said of the anoxemia due to imperfect oxygenation of the arterial blood associated with a low partial pressure of oxygen. Haldane and Priestley (1935b) consider that this difference indicates that the respiratory center responds to the oxygen tension in the arterial blood, which is at its normal level in carbon monoxide anoxemia. The heart is affected, on the other hand, by the reduced quantity of oxygen obtained from the blood in carbon monoxide anoxemia, with the result that exertion causes a fall of blood pressure and faintness. The headache which results from carbon monoxide anoxemia appears to be due to an increased pressure in the cerebro-spinal fluid; there is evidence that such an increase accompanies the headache, and that measures designed to reduce the pressure also relieve the headache (Forbes, Cobb and Fremont-Smith, 1924). These workers record that in one case after the exposure to carbon monoxide, when the headache was worst, ophthalmoscopic examination showed that congestion of the retinal vessels was at a maximum. Forbes et al. (1924) also observed an increased cerebro-spinal fluid pressure, congestion of cerebral vessels, and cerebral edema in dogs and cats during inhalation of carbon monoxide.

Nausea and vomiting are symptoms common to more than one type of anoxemia; they occur in mountain sickness, as the name suggests, and they also occur in carbon monoxide anoxemia. Some degree of nausea is often coexistent with the headache, and vomiting may be precipitated by slight exertion (Killick, 1936).[2] Nausea and vomiting, like the headache, may be at their worst during the recovery period. All these points suggest that the vomiting is due to direct stimulation of the nerve center which controls it, either by the local effects of oxygen lack, or by the edema following the anoxemia.

The symptoms of carbon monoxide anoxemia are strikingly different from those of anemia with a corresponding reduction in hemoglobin; a man whose blood is 50 per cent saturated with carbon monoxide is almost helpless, but a man whose blood contains only 50 per cent of the normal quantity of hemoglobin behaves normally, and can often carry on his work. The partial pressure of oxygen in the arterial blood is

[2] Unpublished observations.

much the same in the two cases, and the quantity of oxygen carried is the same, but the dissociation of oxygen from the oxyhemoglobin is quite different. Douglas, Haldane and Haldane (1912) showed that when the blood is partially saturated with carbon monoxide the dissociation curve of the remaining oxyhemoglobin is altered in such a way that the oxygen dissociates at a much lower partial pressure than usual. This means that the oxygen tension in the tissues must fall much lower than it would if the oxygen dissociation curve were unaltered. J. B. S. Haldane (1912–1913) has published dissociation curves for the oxyhemoglobin remaining in blood that is saturated to various degrees with carbon monoxide which demonstrate this point. Stadie and Martin (1925–1926) made it still clearer by comparing the oxygen dissociation curve of blood containing 40 per cent of the normal quantity of hemoglobin with that of the oxyhemoglobin in blood that was 60 per cent saturated with carbon monoxide. They point out that the alteration in shape and position of the dissociation curve causes a marked lowering of the partial pressure at which oxygen is available for tissue metabolism, and therefore profound anoxemia occurs in carbon monoxide poisoning although the blood may contain 2 or 3 times the amount of oxygen necessary for normal tissue function.

Effects of carbon monoxide anoxemia. There are innumerable clinical records of carbon monoxide poisoning, and a multiplicity of after-effects has been ascribed to it, most of which represent the effect of anoxemia on various parts of the body. The literature on this subject has been summarized by Glaister and Logan (1914b), Sayers and Davenport (1930), and Drinker (1938b).

Central nervous system. The central nervous system is, of course, the tissue most easily damaged by anoxemia, and it shows many changes as a result of carbon monoxide poisoning. After death from carbon monoxide poisoning the brain is found to be hyperemic and edematous, usually showing numerous punctate hemorrhages (Mott, 1907; Hill and Semerak, 1918). Mott (1907) and Hiller (1924) suggest, on the basis of postmortem examination of brains from cases of carbon monoxide poisoning, that these appearances are due to a toxic action of the carbon monoxide on the capillaries. Degeneration of nerve cells is commonly found in the cerebral cortex and in the globus pallidus of the lenticular nucleus (Hill and Semerak, 1918; Hadfield, 1929; Christiani, 1934). Bilateral softening of the globus pallidus is so frequently found as to be characteristic of death from carbon monoxide poisoning; Hill and Semerak found obvious softening in 14 out of 32 cases, and micro-

scopical evidence of degeneration in every case. Meyer (1928) records a symmetrical affection of the pallidum as an extremely regular finding in dogs poisoned with carbon monoxide. Kolisko (1914) put forward an explanation of this localization on the basis of the anatomical arrangement of the arteries to the part, making the pallidum peculiarly liable to anoxemia. Hadfield (1929), in a small series of cases, found that this focal degeneration was associated with vascular siderosis of the pallidum, and concluded that "vascular siderosis predisposes to the acute bilateral destruction of the globus pallidus which is frequent in coal-gas poisoning." Schäffer (1903) and Hsü and Cheng (1938) have drawn attention to necrosis of nerve fibers both in the central nervous system and in peripheral nerves; Hsü and Cheng describe a diffuse reaction in the deeper cerebral white matter which is not uncommon in carbon monoxide poisoning.

An extensive pathological investigation was carried out on dogs by Yant, Chornyak, Shrenk, Patty and Sayers (1934); in dogs exposed to a concentration of carbon monoxide that killed in 20 to 30 minutes the brain showed considerable edema, with dilated blood vessels and some petechial hemorrhages, especially in the cerebral cortex and corpus striatum. The nerve cells were severely damaged, the most affected being those of the cortex, corpus striatum, dorsal motor nuclei of the vagi, and the dorsal sensory areas of the medulla. When the concentration of carbon monoxide was lower, so that the dogs died after 7 to 15 hours, the pathological changes differed only in degree from those observed in animals that survived only 20 to 30 minutes. The edema and the circulatory changes were more marked, and the degenerative changes in the nerve cells had progressed farther, but the areas showing the most severe effects were still the same. The third group of dogs was exposed to a concentration of carbon monoxide that produced a blood saturation of 65 to 75 per cent; this condition was maintained for 15 hours, at the end of which period the dogs appeared moribund. They were then removed from the poisonous atmosphere and allowed to recover; some of the dogs died within a few days and the others were killed at various intervals. There was a great variation in the pathological changes observed in the different animals; the most marked lesions occurred in 2 dogs killed 62 and 65 days after exposure, while the changes exhibited by animals killed before and after these two were less severe. In general the findings in these animals were extensive proliferation of neuroglia and endothelium, large cystic areas in the medullary substance of the brain, with chromatolysis or frag-

mentation of some of the nerve cells; there were also focal areas of myelin degeneration throughout the nervous system, including the peripheral nerves. These results were compared with the effects of simple O_2 lack, and the pathological findings were very similar. This similarity seems to conflict with the view that carbon monoxide has a toxic effect on the capillaries (see p. 324), and suggests that the damage to blood vessels results from oxygen lack.

These experimental findings are in agreement with the pathological changes observed in human beings killed by carbon monoxide. But in spite of these serious changes in the central nervous system permanent mental or neurological disease is a rare sequela of carbon monoxide poisoning; Shillito, Drinker and Shaughnessy (1936) examined the records of 21,143 cases of carbon monoxide poisoning occurring during 10 years in New York, and found only 39 patients suffering from mental or neurological after-effects. All of these 39 patients had been severely poisoned, all were unconscious when found, and 14 of them were described as "in extremis."

Susceptibility to infection. It has often been claimed that carbon monoxide poisoning increases susceptibility to infection. The evidence adduced in support of this statement is that pneumonia frequently follows severe acute carbon monoxide poisoning; this subject is discussed very fully by Glaister and Logan (1914c), who quote an impressive series of recorded deaths from pneumonia after severe carbon monoxide poisoning. Löwy (1925) suggested that as a result of the tissue anoxemia either resistance to infection is lowered, or the virulence of bacteria is raised. Drinker (1938c) is skeptical of the suggestion that carbon monoxide has any specific effect in increasing susceptibility, and considers that the incidence of pneumonia is associated with a long period of unconsciousness, whatever the cause.

It has also been stated that long continued exposure to low concentrations of carbon monoxide predisposes to infection, and Sudhues (1932) states that it is generally accepted that chronic carbon monoxide poisoning causes diminished resistance to infection, but in experimental work on rabbits she could find no difference in the blood content of natural complement, agglutinins, or in phagocytosis, between her controls and animals exposed daily to carbon monoxide over a period of 2 years, nor did she find any difference in the production of specific antibodies to typhoid bacilli. Buresch (1933), on the other hand, did find a loss of hemolytic complement in rabbits exposed daily to carbon monoxide, and also a lowered bactericidal index in dogs similarly

treated. There seems to be little evidence that susceptibility to infection is increased in human beings as a result of repeated exposure to low concentrations of carbon monoxide; Beck (1936) does not mention this as one of the symptoms observed in his series of 97 patients who had been subjected repeatedly to sub-lethal doses of carbon monoxide, and Sayers and his co-workers (1929) record no indication that repeated exposures under experimental conditions were deleterious to the health of their subjects. Löwy (1926), however, cites diminished resistance to infection as one of the effects of chronic exposure to carbon monoxide.

Cardio-vascular system. The heart shows comparatively little after-effect from carbon monoxide anoxemia; during the period of anoxemia the heart rate at rest is unaffected until the saturation of the blood reaches 25 to 30 per cent (Sayers, Yant, Levy and Fulton, 1929; Killick, 1936; Forbes, Dill, de Silva and Van Deventer, 1937). After this the pulse rate increases approximately in proportion to the saturation of the blood with carbon monoxide (Killick, 1936), but as the anoxemia becomes more severe the heart begins to fail. Exercise, when the blood is partially saturated with carbon monoxide, causes an abnormal increase in pulse rate.

Drinker (1938d) considers that individuals with antecedent heart disease are killed immediately as a result of acute poisoning by carbon monoxide, or they survive only a short time, whereas individuals with thoroughly sound hearts may develop chronic heart disease as a sequela of acute poisoning, although this happens only very rarely. It is not infrequent to find the signs of a dilated heart without any organic damage after acute poisoning with carbon monoxide (Zondek, 1919, 1920; Haldane and Priestley, 1935e), and in these cases the heart muscle often recovers completely. Stearns, Drinker and Shaughnessy (1938) took electrocardiograms in 22 cases of acute poisoning, the patients being examined after the period of anoxemia was over, and found changes in the T wave and in the S-T segment in most of the patients, but disturbances of rhythm and conduction were rare. Disturbances of conduction have been produced in animals by severe asphyxia (Lewis, White and Meakins, 1913–1914; Haggard, 1921) but Haggard states that the oxygen deficiency caused by carbon monoxide is not in itself sufficient to cause impairment of auriculo-ventricular conduction, which is only induced by the added anoxemia of respiratory failure.

In recurrent exposure to carbon monoxide, palpitation is a frequent complaint, sometimes accompanied by cardiac pain (Von Dassel, 1932; Beck, 1936), and irregularities of rhythm are also described (Löwy,

1926). Beck (1936) found a slowing of the pulse rate and lowering of blood pressure in 50 per cent of his cases of chronic exposure, but Ciampolini (1924) records a tendency to increased pulse rate under similar conditions. It is not quite clear, however, whether Ciampolini refers to the resting pulse rate, so the discrepancy may be only apparent. In a series of 136 cases of chronic carbon monoxide anoxemia Beck and Suter (1938) found that myocardial symptoms, e.g., anginal attacks, were frequently manifested. As in the production of lesions in the brain, the affection of the heart was primarily vascular, and coronary thrombosis was not infrequent. Argyll Campbell (1929) considers that the ability to tolerate prolonged exposure depends on the response of the heart; he found atrophy of parts of the heart muscle in some of his animals after prolonged exposure to carbon monoxide, and he also describes a hypertrophy of the heart muscle in mice (1932).

Glaister and Logan (1914d) state that during inhalation of carbon monoxide the blood pressure first rises, with a feeling of fulness and throbbing in the head, but later falls to a low level. Brewer (1937) found that in dogs pure carbon monoxide did not produce the rise in blood pressure that breathing nitrogen did, and that carbon monoxide in air caused a fall of blood pressure, but the experimental conditions were so extreme that it is doubtful whether these findings have any significance in relation to the effect of breathing the usual relatively low atmospheric concentrations of carbon monoxide.

A number of cases are on record where a man has been exposed to carbon monoxide, but without very severe immediate symptoms, and yet has collapsed and died some minutes or hours later (Glaister and Logan, 1914e; Drinker, 1938e); such sudden death is usually cardiac in origin, and is of some medico-legal interest, for death may occur under circumstances suggesting foul play.

The reference to thrombosis in the coronary vessels, and also in the cerebral vessels, is interesting in view of the old controversy as to whether the blood is more fluid than usual after death from carbon monoxide. It is often claimed that the blood is abnormally fluid at death, and does not readily coagulate (Glaister and Logan, 1914f; Ramsey and Eilmann, 1931), while on the other hand Rambousek (1913) states that carbon monoxide favors coagulation. Forbes and Hompe (1921) attempted to obtain evidence on this point by measuring the clotting time of the blood in cats poisoned fairly rapidly with carbon monoxide; they found no alteration in clotting time and no change in prothrombin content. It seems likely that the hemorrhages

and thrombi found after fatal carbon monoxide anoxemia are due to changes in the capillary walls rather than in the blood itself (Drinker, 1938f). Semerak and Bacon (1930) have summarized the literature dealing with changes in the vessel walls, and conclude that various degenerative changes may follow severe carbon monoxide anoxemia.

Metabolism. Carbon monoxide anoxemia, as would be expected, affects the metabolism in several ways. Walters (1927) showed that in white rats continuously exposed to low concentrations of carbon monoxide the metabolic rate was diminished even in the early stages of anoxemia (16 to 35 per cent saturation of the blood), and that this fall in metabolic rate could be correlated with the symptoms of poisoning. Haldane (1895a) demonstrated the same effect in mice. On the other hand Schulze (1936a), using white mice, and Reploh (1932), using rabbits, observed that repeated exposure to carbon monoxide caused an increase in metabolic rate. Schulze (1936a) describes changes in the thyroid in his animals of a type usually associated with increased activity. Kampelmann and Schulze (1937) extended this investigation by observing that in guinea pigs repeatedly exposed to carbon monoxide there was a diminution of the thyrotropic hormone of the pituitary, which appeared to be secondary to the activation of the thyroid. Yet a third type of result is that recorded by Haggard and Henderson (1921); they found that when dogs were rapidly gassed the oxygen consumption did not fall, but either remained unaltered or increased. They attributed the increase, when it occurred, to the increased activity of the respiratory muscles involved in the considerable degree of hyperpnea that was associated with the anoxemia.

Summing up these experimental findings, it seems that during exposure to low concentrations of carbon monoxide, when the anoxemia develops slowly, and is not so severe as to cause collapse and death, the metabolic rate is slowed; when the anoxemia is rapid and severe, as in Haggard and Henderson's experiments, this effect does not develop and the metabolic rate may actually rise as a result of increased activity of the respiratory muscles. Repeated severe poisonings, as in Reploh and Schulze's experiments, appear to affect the ductless glands in such a way that the metabolism is increased; this increase, it must be emphasized, was observed between or following the successive periods of anoxemia and not during these periods, as was the case in Haldane's and Walters' experiments. The only relevant observation on human subjects seems to be that of Beck (1936) who found that the metabolic rate was significantly reduced in just over half of his patients who had

been repeatedly exposed to carbon monoxide, and secondly, the observation frequently made that in acute poisoning there is a very marked fall of body temperature (Drinker, 1938g), presumably due to depressed metabolism.

Several workers have recorded an increased urinary excretion of total nitrogen and of NH_3 in carbon monoxide poisoning (Glaubitz, 1921; Tscherkess and Melnikova, 1928). Tscherkess and Melnikova subjected various animals to short periods of severe carbon monoxide anoxemia, and found that both urinary nitrogen and inorganic phosphorus were increased during and after the poisoning, returning to normal after 1 to 3 days. It is claimed that this indicates increased destruction of protein, and a raised metabolic rate, but apparently no other method of estimating the metabolic rate was employed.

Glycosuria often occurs as a result of carbon monoxide anoxemia, as was first described by Claude Bernard (1857), and seems to be due to the mobilization of the liver glycogen, as it does not occur when starved animals that have lost their liver glycogen are subjected to anoxemia (Araki, 1891). Kellaway (1919) considers that the mobilization of sugar is an effect of anoxemia on the central nervous system, although accelerated output of adrenin may play a part. Schulze (1936b), in his experiments on mice, found that the blood sugar rose steeply as a result of each exposure to carbon monoxide, and returned gradually to normal in about 3 hours; the liver glycogen diminished with successive exposures, indicating the source of the blood sugar. Buresch (1933) also observed an increased blood sugar in rabbits exposed daily to carbon monoxide, although Boedicker (1933) could find no definite evidence that these repeated exposures altered the form of the blood sugar curve following a dose of glucose by stomach tube. Mikami (1926–27) records that in rabbits the rise of blood sugar resulting from administration of carbon monoxide was proportional to the degree of anoxemia; he also found that intravenous injection of alkali inhibited the hyperglycemia, and also prevented the fall in arterial carbon dioxide content associated with acute poisoning (see p. 331). This connection suggests that changes in the acid-base balance of the blood may play a part in the production of hyperglycemia.

Respiration and chemical changes in the blood. A number of conflicting views have been expressed as to the effect of carbon monoxide anoxemia on the acid-base equilibrium of the blood, but most observers have found a decreased alkali reserve (Haggard and Henderson, 1921; Mikami, 1926–27; Tscherkess and Melnikova, 1928; Kamei, 1931),

although their interpretations of this result vary. Haggard and Henderson exposed dogs to 0.25 per cent carbon monoxide; during the period of anoxemia the respiration increased greatly, the volume sometimes being three times as great as before the experiment started, the carbon dioxide combining power of the arterial blood fell gradually while its carbon dioxide content fell more rapidly. The hyperpnea was prevented by section of both vagi. From these results they concluded that the hyperpnea caused by anoxemia produced an alkalosis by washing out carbon dioxide, and that the alkali reserve fell as a result of the alkalosis. The absence of hyperpnea after vagal section they regard as evidence that "oxygen deficiency by itself does not directly cause in the tissues and blood an increased production of organic acids." Neither Mikami nor Kamei, however, was able to confirm this effect of vagal section. Both these workers used hypodermic injection as a method of administering carbon monoxide; Mikami observed a very marked fall in arterial carbon dioxide content in rabbits, but since he only measured the respiratory rate, and not the volume, his statement that the increased breathing was not sufficient to account for the fall in carbon dioxide is not very convincing: he found that the plasma pH was definitely lowered as a result of carbon monoxide anoxemia. Kamei also observed an increased ratio of dissolved to combined carbon dioxide in the blood of dogs similarly treated. Mikami and Kamei both administered carbon monoxide by inhalation as well as by injection, and claim that the results were very similar. Haggard and Henderson noticed in their dogs that during the early recovery period the respiration was depressed, with resultant retention of carbon dioxide, and development of acidosis. It seems possible that the diminished pH observed by Mikami and Kamei was actually recorded during such a period of depressed respiration, especially as it could not be easy to determine the stage of maximum anoxemia after injection of carbon monoxide.

In human beings hyperpnea does not seem to be such a prominent symptom of carbon monoxide poisoning; Haldane (1895b) in experiments on himself noted that hyperpnea began to appear when his blood was about 30 per cent saturated with carbon monoxide, and became more marked as the saturation increased. Sayers and his co-workers (1929) state that in their series of experiments on human subjects there was no change in the respiration as a result of exposure to 0.02 to 0.04 per cent carbon monoxide. It is interesting that Kamei (1931) states that in his dogs inhalation of 1 per cent carbon monoxide

caused hyperpnea, whereas inhalation of 0.05 to 0.08 per cent did not; in the experiments referred to above when Haldane experienced hyperpnea he was breathing fairly high concentrations of carbon monoxide (0.12 per cent–0.50 per cent), while Sayers' subjects only breathed low concentrations. Unfortunately in these human experiments no estimations were made of carbon dioxide combining power or carbon dioxide content of the blood, but the available evidence supports the hypothesis that the diminished alkali reserve is associated with hyperpnea, and is strictly comparable with the change caused by the anoxemia of high altitude.

Reproduction and sex glands. Carbon monoxide anoxemia seems to affect the gonads; McCombs (1912) records that men surviving acute poisoning are sometimes impotent, and Drinker (1938h) has seen such a case; Rossiter (1928) refers to loss of sexual desire after continual exposure to carbon monoxide in sub-lethal doses, and Williams (1930) states that poisoning may cause death and expulsion of the fetus. Nicloux (1901), in his work on the passage of gases across the placenta, records that during slow poisoning of pregnant guinea pigs with carbon monoxide the fetal and maternal bloods contained the same quantity of carbon monoxide, but that when exposed to a high concentration of carbon monoxide the guinea pigs died before an appreciable amount of carbon monoxide had reached the fetal blood, although the fetus suffered from deprivation of oxygen.

There is considerable experimental evidence that prolonged exposure to low concentrations of carbon monoxide greatly reduces fertility in animals (Campbell, 1934 (mice); Williams and Smith, 1935 (rats); Patterson, Smith, and Pickett, 1938 (mice)). Buresch (1933), working with rabbits and mice, found that repeated exposures to carbon monoxide caused a tendency for the females to abort. Williams and Smith used a concentration of carbon monoxide sufficient to produce 60 to 70 per cent saturation of the blood in the course of an hour; daily exposures affected the females so that they first produced inferior offspring, later dead feti, and finally pregnancies ceased. The males after 75 daily exposures were apparently sterile, the sperm cells being non-motile or absent; the testes were reduced to $\frac{1}{3}$ to $\frac{1}{2}$ the weight of the controls. These results were confirmed by Patterson, Smith and Pickett, who also observed that in the males the hypophysis became highly basophilic and contained many vacuolated cells (such as are seen after castration); in addition they demonstrated an increase in the content of gonadotropic hormone. It should be noted that Williams and Smith, and

Patterson and his co-workers used illuminating gas as a source of carbon monoxide; this gas has been shown to be somewhat more toxic than its content of carbon monoxide indicates (Haggard, 1922; Henderson, Haggard, Teague, Prince and Wunderlich, 1921b), and it is possible that this may account in part for their results.

Acclimatization. Certain adverse effects of continuous or repeated exposure to carbon monoxide have already been described, but another result of such treatment must now be discussed; the response of the healthy man or animal is to develop some degree of acquired tolerance of the gas, although adverse effects may show themselves at the same time. This acquired tolerance or acclimatization to carbon monoxide has been recognized for a considerable period among those whose work exposes them to carbon monoxide (Faure, 1856; Glaister and Logan, 1914g; Oliver, 1916; Official History of the War, 1923), and its occurrence has been confirmed more recently by experimental methods.

The first systematic attempt to find an explanation of the phenomenon was made by Nasmith and Graham (1906). They kept guinea pigs continuously in an atmosphere that produced a 25 per cent saturation of their blood with carbon monoxide; after the first few days the animals appeared quite normal, but the red cell count and the hemoglobin content of their blood gradually rose in the course of 3 to 4 weeks from an average value of 5.88 million red cells and 88 per cent hemoglobin, to 7.96 million red cells and 105 per cent hemoglobin. The concentration of carbon monoxide in the air was then increased to give 35 per cent saturation of the blood, and the red cell counts rose again to an average of 9 million. Finally the saturation of the blood was put up to 45 per cent and the animals at first showed symptoms of poisoning, but they recovered in a few days and the red cell count eventually reached an average of 10.5 million with a hemoglobin index of 110 per cent. The degree of acclimatization was indicated by the observation that fresh guinea pigs placed in this atmosphere only survived a few days, whereas the acclimatized animals appeared to be unaffected. Nasmith and Graham record that degenerative changes in the red cells preceded the erythrocytosis, and that some degree of eosinophilia was observed. These experiments yielded no evidence as to whether the percentage saturation of the blood with carbon monoxide altered as acclimatization developed.

Argyll Campbell (1929–1930, 1933, 1934) carried the investigation of the problem a stage further, and showed that rabbits, rats, mice and guinea pigs could tolerate a much higher atmospheric concentration

of carbon monoxide after acclimatization by continuous exposure than they could when unacclimatized. He confirmed in these animals the observation of polycythemia made by Nasmith and Graham. He attempted to follow the progress of acclimatization by estimating the tensions of oxygen and carbon monoxide in the tissues during exposure of rabbits to carbon monoxide. The tension of carbon monoxide maintained a fairly constant relationship with the concentration of the gas in the air breathed as acclimatization developed, but in the acclimatized animals the tissue oxygen tension did not fall quite so low as it did in the unacclimatized when both had their blood saturated to the same extent with carbon monoxide; this was probably a result of the increased oxygen capacity of the blood. Campbell's data for the percentage saturation of the blood with carbon monoxide indicate that its relationship with the atmospheric concentration of carbon monoxide remained unaltered as acclimatization developed. Campbell (1934) considers that a strict criterion of acclimatization should be adopted, and requires evidence of the maintenance of growth, body weight, appetite, general well-being and fertility. Perhaps the term "tolerance" should be used to describe any alteration short of this complete acclimatization, but it is usual to describe the disappearance of positive symptoms of poisoning as acclimatization.

Killick (1937) confirmed these observations on the effects of continuous exposure of mice to carbon monoxide. She found a considerable polycythemia, with a marked increase in the proportion of reticulocytes; there was enlargement of the spleen and some evidence of an increased blood volume. The reaction of the spleen is interesting, since there is evidence that this organ plays a considerable part in acute poisoning. Barcroft and Barcroft (1923) and Hanak and Harkavy (1924) have recorded a discrepancy between the carbon monoxide content of the spleen blood and of the circulating blood during exposure of rats and guinea pigs. The spleen blood apparently takes up carbon monoxide more slowly than the blood in the general circulation, and the time taken for the spleen pulp to attain equilibrium with the blood varied up to 6 hours.

De Boer and Carroll (1924–25) showed that in cats the spleen contracted when the animals were exposed to carbon monoxide, the contraction beginning when the percentage saturation of the blood with carbon monoxide was quite low. This contraction would presumably have the effect of expelling into the circulation blood from the spleen pulp whose carbon monoxide content was lower than that of the cir-

culating blood. That this mechanism is a useful one was shown by Barcroft, Murray, Orahovats, Sands, and Weiss (1925), when they compared the reaction of normal and splenectomized guinea pigs to carbon monoxide. The splenectomized animals succumbed in $\frac{3}{4}$ of the time of the normal animals.

It is not easy to see how this train of events could be of use when the animals live in an atmosphere containing carbon monoxide, but the enlargement of the spleen observed by Killick may have been connected with the polycythemia and increased blood volume. Muira's suggestion (1936) that the spleen removes from the circulation the erythrocytes containing carboxyhemoglobin is difficult to reconcile with the observation of a lag in the percentage saturation of the spleen pulp behind that of the circulating blood. It also assumes, apparently, that a red cell takes up oxygen or carbon monoxide, but not both, an assumption which hardly seems justified.

In the course of their estimations of the arterial oxygen tension by the carbon monoxide method Douglas and Haldane (1912) found that when the blood of mice became more than about 30 per cent saturated with carbon monoxide the arterial oxygen tension began to rise above that of the alveolar air, and Haldane and Priestley (1935f) consider that this power of secreting oxygen into the blood explains some of the phenomena of acclimatization to anoxemia. Killick (1937), in her experiments on mice, attempted to test this hypothesis by comparing the percentage saturation of the blood in vivo with the percentage saturation reached when the blood of the same animal was equilibrated in vitro with mixtures of air and carbon monoxide. The equilibration of several samples of blood from the same mouse provided the data for the calculation of the equilibrium constant, and when this value was known the equation

$$\frac{[HbCO]}{[HbO_2]} = \frac{K[CO]}{[O_2]}$$

could be used to calculate the arterial oxyten tension during life. The arterial oxygen tensions in the acclimatized mice, calculated in this way, fell within limits corresponding with the alveolar oxygen tension one might expect to find in mice. The conclusion was that under the conditions of the experiment acclimatization to carbon monoxide was not accompanied by any change in the absorption of carbon monoxide in the lungs, due to the secretion of oxygen.

Summing up the results of animal experiments, therefore, one can

state that animals develop a considerable degree of acclimatization to carbon monoxide, as a result of continuous or repeated exposure to low concentrations of the gas. This acclimatization is accompanied by an increased production of erythrocytes, thus raising the count in the circulating blood, and in certain modifications in the circulation, indicated by hypertrophy of the heart (Campbell, 1932), increased blood volume, and splenic changes. There is no evidence of any change in the relative affinity of hemoglobin for carbon monoxide (Killick, 1937), and the evidence as to some active process in the alveolar epithelium, such as secretion of oxygen, is conflicting.

Attempts to acclimatize human subjects by experimental methods have yielded somewhat conflicting results. Haldane (Haldane and Priestley, 1935g) records that he and Lorrain Smith became acclimatized during the course of a series of experiments in which they breathed carbon monoxide as a method of determining the arterial oxygen tension (Haldane and Smith, 1896). After a number of experiments they found it was necessary to breathe 0.06 per cent of carbon monoxide in order to produce the desired blood saturation of 30 per cent. Theoretically this concentration should produce about 55 per cent saturation of the blood; after a considerable lapse of time 0.04 per cent carbon monoxide was inhaled, and very severe symptoms were produced. Evidently the subject was acclimatized when breathing 0.06 per cent carbon monoxide, but had lost this acclimatization in the later experiment. The interesting point about this observation is that as a result of acclimatization there was a marked alteration in the relationship between the concentration of carbon monoxide inhaled and the percentage saturation of the blood.

Sayers, Yant, Levy and Fulton (1929) investigated the effect of repeated daily exposures to small amounts of automobile exhaust gas; the exhaust gas was used as a source of carbon monoxide and its concentration was adjusted to give the required concentration of carbon monoxide. The investigation was planned to give information as to the effects of exposure to exhaust gas such as traffic police and other officials would encounter when on duty in a road tunnel, and the observations made are so arranged as to make it difficult to conclude with certainty whether or not any acclimatization resulted. The six subjects were confined in an air-tight chamber of 1000 cu. ft. capacity for a period of 5 to 6 hours every day, and a continuous change of air was maintained during this time. Exhaust gas was added to the air in amounts sufficient to produce a concentration of 0.02 per cent, 0.03

per cent, or 0.04 per cent of carbon monoxide, the concentration being maintained constant throughout any one exposure. Control tests were interspersed amongst the carbon monoxide tests, in which no exhaust gas was added to the air.

The saturation of the blood with carbon monoxide was followed by taking blood samples at intervals from two of the subjects each day, and these results are recorded altogether, without any statement as to the subject whose blood was being examined, or the serial number of the exposure. After 8 control tests had been made 15 successive tests were carried out with 0.02 per cent carbon monoxide; during these tests the three subjects at rest experienced frontal headache towards the end of the exposure (in 22 per cent of the maximum number of occasions), while the other three, who were exercising moderately, experienced frontal headache slightly more frequently (28 per cent of the possible total). The recorded blood saturations are plotted against time of exposure on a single graph; the points are widely scattered, but since there is no indication of the source of the blood or the test during which it was taken one cannot judge whether the scatter represents the experimental error, the variation between the subjects, or the development of acclimatization. The lines which are drawn to represent the average blood saturation are of such a shape as to throw some doubt on the reliability of the method of estimating blood saturation (pyrotannic acid method). Two series of tests were made using 0.04 per cent carbon monoxide, and then a final series with 0.03 per cent carbon monoxide. In this final series the symptoms recorded were intermediate in severity between the exposures to 0.02 per cent and those to 0.04 per cent, which suggests that no acclimatization had occurred. The blood saturation attained in this final series corresponds fairly closely to the theoretical value. The effect of exercise was to hasten the absorption of carbon monoxide, and to increase the severity of the symptoms experienced.

During this series of exposures, extending over two months, the hemoglobin content of the blood of 5 of the 6 subjects increased, while the red cell count also increased in 4 subjects; the greatest increase was 20 per cent in the hemoglobin content and 1,000,000 in red cells.

There was no evidence of any deleterious effect on the health of any of the subjects.

A somewhat similar series of experiments, but performed on a single subject, was undertaken by Killick (1936) in an attempt to induce acclimatization under controlled conditions. The subject was exposed

to an atmosphere containing a low concentration of pure carbon monoxide in an air-tight chamber. The exposures were repeated at intervals of 4 to 7 days over a period of 3 to 4 months. The subject developed a considerable degree of acclimatization, as evidenced by the symptoms experienced. The blood saturation was followed during each exposure and it was found to increase in a regular manner, 25 per cent, 50 per cent and 75 per cent of the final equilibrium value being reached at approximately the same period of exposure, whatever the atmospheric concentration of carbon monoxide (see p. 317, fig. 1). The relationship between the final equilibrium value and the atmospheric concentration of carbon monoxide, however, altered as acclimatization developed. The extent of this alteration is indicated by the fact that the equilibrium value for the percentage saturation of the blood in the unacclimatized subject during exposure to 0.02 per cent carbon monoxide was the same as that observed during exposure of the acclimatized subject to 0.04 per cent carbon monoxide; furthermore the symptoms experienced on the two occasions were very similar. The value of the equilibrium constant for the subject's blood was determined in vitro, and was found to remain unaltered as acclimatization developed. These results, therefore, were due to some changed response of the living body, and two possible explanations were considered: 1, a selective activity of the alveolar membrane producing either a secretion of oxygen from the alveoli into the blood, or a hindrance to diffusion of carbon monoxide inwards; 2, removal of carbon monoxide from the blood by oxidative or other processes in the tissues.

The second explanation is improbable, since there is no evidence that the body can oxidize carbon monoxide (see p. 320). There are difficulties, also, in accepting the hypothesis of oxygen secretion; it has been claimed that the stimulus to oxygen secretion is an oxygen deficiency in the tissues (Haldane and Priestley, 1935h), but these experiments showed that the process underlying acclimatization was active when the blood was only 10 to 15 per cent saturated with carbon monoxide, a degree of saturation that is unlikely to be associated with oxygen lack in the tissues while the subject is at rest. This suggests that the explanation may be found in some process that is stimulated by the presence of carbon monoxide, in the lungs or elsewhere.

In these experiments Killick observed no increase in hemoglobin or in the number of red cells, but it must be remembered that exposures to carbon monoxide were not very frequent.

There is some evidence that acclimatization in monkeys may be of

the same type as that described by Killick in the human subject. Van Bogaert, Dallemagne and Wégria (1938) exposed a monkey (Macacus rhesus) daily to a concentration of carbon monoxide sufficient to produce 30 per cent saturation of its blood in the course of an hour. The actual degree of saturation produced varied somewhat from day to day, probably as a result of the varying activity of the animal, but it oscillated about 30 per cent during the 6 months of the experiment. At first the atmospheric concentration necessary to give 30 per cent saturation was 0.01 per cent carbon monoxide, but in the course of six months the concentration had to be raised gradually to 0.15 per cent carbon monoxide in order to maintain a blood saturation of about 30 per cent. It is interesting that during this experiment neither the oxygen capacity nor the red cell count of the blood altered appreciably.

There is considerable clinical evidence of an increase in hemoglobin and red cell count in the blood of men whose work involves regular or frequent exposure to carbon monoxide (Karasek, 1909–1912; Beck, 1927, 1936; Jenkins, 1932). Red cell counts as high as 9.68 millions have been recorded (Karasek) but usually the hemoglobin index does not show as great a rise. Anemia is also recorded as resulting from chronic poisoning (Beck and Fort, 1924–25; Biedermann, 1938), but it seems possible that in such cases there has been exposure to other toxic gases in addition to carbon monoxide. In the same way the slight increase in fragility of the red cells that has been attributed to carbon monoxide (Williams and Smith, 1935) is probably caused by some other constituent of illuminating gas (Mayers, Rivkin, Krasnow, 1930).

It appears that this increase in the oxygen carrying capacity of the blood, associated with acclimatization in man as in animals, may be supplemented, under certain conditions, by the change in the degree of saturation of the blood already described, so that the blood of the acclimatized individual attains a lower percentage saturation than that of the unacclimatized, when both individuals are exposed to the same atmospheric concentration of carbon monoxide.

In spite of the development of acclimatization there is little doubt that chronic exposure to low concentrations of carbon monoxide does cause ill-health. Reference has already been made to some of the symptoms of this condition, and the commoner symptoms may be listed as follows: Headache, dizziness, drowsiness, digestive disturbances, dyspnea, palpitation, muscular weakness (Mayers, 1930; Beck, 1936).

Treatment. The treatment of acute carbon monoxide poisoning is directed toward shortening the period of anoxemia by hastening the

elimination of carbon monoxide from the blood. Hill and Flack (1908) were the first to suggest the use of carbon dioxide for this purpose, and Henderson and Haggard (1920 and 1922) demonstrated the efficiency of carbon dioxide and oxygen mixtures by experimental work on dogs and later in a series of human poisoning cases. Sayers and Yant (1923) found that the rate of elimination of carbon monoxide from the blood was represented by the equation:

$$\text{Log } S' = \log S + (b \log e)\ t$$

where S was the initial saturation, S' the saturation after time t, and $(b \log e)$ was a constant; they found that the value of this constant varied with the treatment employed, the rate of elimination being fastest with a mixture of about 10 per cent carbon dioxide in oxygen, slowest with air, and intermediate when pure oxygen was administered. Stadie and Martin (1925) in their experiments on dogs obtained curves for the rate of elimination of carbon monoxide which conformed to the above equation, and they also showed that the effect of carbon dioxide in lowering the pH of the blood was an important factor in its action.

It appears from Henderson and Haggard's work (1922), however, that this equation does not represent the rate of elimination in the early stages of recovery from severe anoxemia; in these cases the breathing is at first depressed, and in the absence of treatment elimination is very slow. This additional factor emphasizes the importance of administering carbon dioxide to stimulate the breathing.

Various additional forms of treatment have been recommended from time to time, but most of them are useless, if not actually harmful; e.g., blood transfusion, bleeding, injection of methylene blue. The latter suggestion (Barron et al., 1928a, b; 1930, 1932; Brooks, 1932) is based on the effect of methylene blue in stimulating oxygen consumption independently of the tissue enzyme systems, but Haggard and Greenberg (1933) and Nadler, Green and Rosenbaum (1934) have pointed out that methylene blue converts hemoglobin into methemoglobin, and therefore must increase the degree of anoxemia. Haggard and Greenberg in experiments on animals demonstrated methylene blue to be useless or even dangerous in carbon monoxide anoxemia. Henderson and Haggard (1922) consider that if oxygen and carbon dioxide are administered, together with artificial respiration if this is necessary, the less the patient is disturbed by other forms of treatment the greater his chances of complete recovery. The only effective treatment of chronic carbon monoxide anoxemia is to put an end to the exposure to the gas.

REFERENCES

ANSON, M. L., J. BARCROFT, A. E. MIRSKY AND S. OINUMA. Proc. Roy. Soc. B. **97**: 61, 1924–1925.

ARAKI, T. Ztschr. f. Physiol. Chem. **15**: 335, 1891.

BARCROFT, J. AND H. BARCROFT. J. Physiol. **58**: 139, 1923.

BARCROFT, J., C. D. MURRAY, D. ORAHOVATS, J. SANDS AND R. WEISS. J. Physiol. **60**: 79, 1925.

BARKAN, G. Deutsch. med. Wchnschr. **64**: 638, 1938.

BARRON, E. S. G. AND M. HAMBURGER. J. Biol. Chem. **96**: 299, 1932.

BARRON, E. S. G. AND G. A. HARROP. J. Biol. Chem. **79**: 65, 1928a.
J. Biol. Chem. **81**: 445, 1928b.

BARRON, E. S. G. AND L. A. HOFFMAN. J. Gen. Physiol. **13**: 483, 1930.

BECK, H. G. Ann. Clin. Med. **5**: 1088, 1927.
J. A. M. A. **107**: 1025, 1936.

BECK, H. G. AND W. FORT. Ann. Clin. Med. **3**: 437, 1924–25.

BECK, H. G. AND G. M. SUTER. J. A. M. A. **110**: 1982, 1938.

BERNARD, C. Lecons sur les effets des substances toxiques et médicamenteuses. Paris, 1857.

BIEDERMANN, H. Folia Haemat. **61**: 186, 1938.

BOEDICKER, W. Arch. f. Hyg. **109**: 124, 1933.

DE BOER, S. AND D. C. CARROLL. J. Physiol. **59**: 312, 1924–1925.

VAN BOGAERT, L., M. J. DALLEMAGNE AND R. WÉGRIA. Arch. Intern. de med. exper. **13**: 335, 1938.

BREWER, N. R. Am. J. Physiol. **120**: 91, 1937.

BROOKS, M. M. Am. J. Physiol. **102**: 145, 1932.

BURESCH, H. Arch. f. Hyg. **109**: 211, 1933.

BURRELL, G. A., F. M. SEIBERT AND I. W. ROBERTSON. U. S. Bur. Mines. Tech. Paper no. 62, 1914.

CAMPBELL, J. A. Brit. J. Exper. Path. **10**: 304, 1929.
J. Physiol. **68**: 81, 1929–1930.
J. Physiol. **77**: 8P, 1932.
Quart. J. Exper. Physiol. **24**: 271, 1934.

CHRISTIANI, E. Med. Welt. **8**: 1469, 1934.

CIAMPOLINI, E. J. Ind. Hyg., **6**: 102, 1924.

VON DASSEL, D. Die Gasmaske **4**: 5, 1932.

DOUGLAS, C. G. AND J. S. HALDANE. Skand. Arch. f. Physiol. **25**: 169, 1911.

DOUGLAS, C. G. AND J. S. HALDANE. J. Physiol. **44**: 305, 1912.

DOUGLAS, C. G., J. S. HALDANE AND J. B. S. HALDANE. J. Physiol. **44**: 275, 1912.

DRINKER, C. K. Carbon monoxide asphyxia, Oxford, 1938.
(a) Chap. II.
(b) Chap. I, II, V.
(c) p. 71; (d) p. 51; (e) p. 50; (f) p. 124; (g) p. 30; (h) p. 54.

FAURE. Arch. Generales de Med. **7**: (5th series): 20, 1856.

FENN, W. O. AND D. M. COBB. Am. J. Physiol. **102**: 379, 1932a.
Am. J. Physiol. **102**: 393, 1932b.

FORBES, H. S., S. COBB AND F. FREMONT-SMITH. Arch. Neurol. and Psychiat. **11**: 264, 1924.

FORBES, H. S. AND L. HOMPE. J. Ind. Hyg. **3**: 213, 1921.

FORBES, W. H., D. B. DILL, H. DE SILVA AND F. M. VAN DEVENTER. J. Ind. Hyg. **19:** 598, 1937.
GLAISTER, J. AND D. D. LOGAN. Gas poisoning in mining and other industries. Edinburgh, 1914.
(a) Chap. VI, IX and X.
(b) Chap. VI, VII, IX, X, XI, XIV.
(c) Chap. VII.
(d) p. 95; (e) p. 101; (f) p. 340; (g) p. 209.
GLAUBITZ, G. Ztschr. f. d. ges. exper. med. **25:** 230, 1921.
GSCHEIDLEN, R. Unters. aus. dem physiol. Lab. Wurtzburg Pt. 2, 154, 1869.
HADFIELD, G. J. Path. and Bact. **32:** 135, 1929.
HAGGARD, H. W. Am. J. Physiol. **56:** 390, 1921.
Am. J. Physiol. **60:** 244, 1922.
HAGGARD, H. W. AND L. A. GREENBERG. J. A. M. A. **100:** 2001, 1933.
HAGGARD, H. W. AND Y. HENDERSON. J. Biol. Chem. **47:** 421, 1921.
HALDANE, J. B. S. J. Physiol. **45:** xxii Proc., 1912–13.
Biochem. J. **21:** 1068, 1927.
HALDANE, J. S. J. Physiol. **18:** 201, 1895a.
J. Physiol. **18:** 430, 1895b.
J. Physiol. **25:** 225, 1899–1900.
HALDANE, J. S. AND J. G. PRIESTLEY. Respiration. Oxford, 1935.
(a) p. 339; (b) p. 239; (c) p. 203; (d) p. 241; (e) p. 204; (f) p. 293; (g) p. 244; (h) p. 272.
HALDANE, J. S. AND J. L. SMITH. J. Physiol. **20:** 497, 1896.
J. Physiol. **22:** 231, 1897.
HANAK, A. AND J. HARKAVY. J. Physiol. **59:** 121, 1924.
HARTRIDGE, H. J. Physiol. **44:** 1, 1912a.
J. Physiol. **44:** 22, 1912b.
HARTRIDGE, H. AND F. J. W. ROUGHTON. Proc. Roy. Soc. B. **94:** 336, 1923.
HENDERSON, Y. AND H. W. HAGGARD. J. Pharmacol. and Exper. Therap. **16:** 11, 1920.
J. A. M. A. **79:** 1137, 1922.
HENDERSON, Y., H. W. HAGGARD, M. C. TEAGUE, A. L. PRINCE AND R. M. WUNDERLICH. J. Ind. Hyg. **3:** 79, 1921a.
J. Ind. Hyg. **3:** 137, 1921b.
HILL, E. AND C. B. SEMERAK. J. A. M. A. **71:** 644, 1918.
HILL, L. AND M. FLACK. J. Physiol. **37:** 77, 1908.
HILLER, F. Ztschr. f. d. ges. Neurol. u. Psychiat. **93:** 594, 1924.
HSÜ, Y. K. AND Y. L. CHENG. Brain **61:** 384, 1938.
JENKINS, C. E. J. Hyg. **32:** 406, 1932.
KAMEI, B. Tohoku J. Exper. Med. **17:** 127, 1931.
KAMPELMANN, F. AND E. SCHULZE. Klin. Wchnschr. **16:** 427, 1937.
KARASEK, M. Trans. Chicago Path. Soc. **8:** 173, 1909–1912.
KELLAWAY, C. H. J. Physiol. **53:** 211, 1919–1920.
KILLICK, E. M. J. Physiol. **87:** 41, 1936.
J. Physiol. **91:** 279, 1937.
KOLISKO, A. Beitr. z. gerichtl. Med. **2:** 1, 1914.
KOZA, F. Bratislavske Lekarske Listy **9:** 16, 1929.

KROGH, A. Skand. Arch. f. Physiol. **23:** 217, 1909–1910.
KROGH, M. Pflüger's. Arch. **162:** 94, 1915.
LEWIS, T., P. D. WHITE AND J. MEAKINS. Heart **5:** 289, 1913–1914.
LÖWY, J. Wien. med. Wchnschr. **75:** 1694, 1925.
Zentralbl. f. Gewerbehyg. **13:** 153, 1926.
MCCOMBS, R. S. Am. J. Med. Sci. **144:** 377, 1912.
MAYERS, M. R. Carbonic monoxide poisoning in industry. Dept. of Labor, State of New York, Bull. 1930.
MAYERS, M. R., H. RIVKIN AND F. KRASNOW. J. Ind. Hyg. **12:** 300, 1930.
DE MEIO, R. H., M. KISSIN AND E. S. G. BARRON. J. Biol. Chem. **107:** 579, 1934.
MEYER, A. Ztschr. f. d. ges. Neurol. u. Psychiat. **112:** 187, 1928.
MIKAMI, S. Tohoku J. Exper. Med. **8:** 237, 1926–1927.
MILLIKAN, G. A. Proc. Roy. Soc. B. **123:** 218, 1936.
MIURA, H. Tohoku J. Exper. Med. **29:** 586, 1936.
MOTT, F. W. Arch. Neur. (from Path. Lab. London County Asylums, Claybury, Essex) III, London 1907, p. 247.
NADLER, J. E., H. GREEN AND A. ROSENBAUM. Am. J. Med. Sci. **188:** 15, 1934.
NASMITH, G. G. AND D. A. L. GRAHAM. J. Physiol. **35:** 32, 1906–1907.
NICLOUX, M. C. R. de l'Acad. des. Sc. **183:** 67, 1901.
Official History of the War. Medical Services, Diseases of the War. Vol. 2, London, 1923.
OLIVER, T. Diseases of occupation. p. 68. London 1916 (3rd ed.).
PATTERSON, C. A., E. SMITH AND A. D. PICKETT. Proc. Soc. Exper. Biol. and Med. **38:** 455, 1938.
RAMBOUSEK, J. Industrial poisoning. Arnold, 1913.
RAMSEY, T. L. AND H. J. EILMANN. J. Lab. and Clin. Med. **17:** 415, 1931–32.
REPLOH, H. Arch. f. Hyg. **107:** 283, 1932.
ROSSITER, F. S. Carbon monoxide poisoning. Carnegie Steel Co. Publ. Pittsburgh, 1928.
ROUGHTON, F. J. W. Proc. Roy. Soc. B. **115:** 451, 1934.
SAYERS, R. R. AND S. J. DAVENPORT. U. S. Public Health Service, Pub. Health. Bull. no. 195, 1930.
SAYERS, R. R. AND W. P. YANT. Pub. Health Rep. **38:** 2053, 1923.
SAYERS, R. R., W. P. YANT, E. LEVY AND W. B. FULTON. U. S. Public Health Service, Pub. Health Bull. no. 186, 1929.
SCHÄFFER. Centralbl. f. Nervenh. u. Psychiat. **26:** (**14** new series): 485, 1903.
SCHULZE, E. Arch. f. Exper. Path. u. Pharmakol. **180:** 639, 1936a.
Arch. f. Exper. Path. u. Pharmakol. **180:** 649, 1936b.
SEMERAK, C. B. AND L. H. BACON. Arch. Path. **10:** 823, 1930.
SENDROY, J., S. H. LIU AND D. D. VAN SLYKE. Am. J. Physiol. **90:** 511, 1929.
SHILLITO, F. H., C. K. DRINKER AND T. J. SHAUGHNESSY. J. A. M. A. **106:** 669, 1936.
VAN SLYKE, D. D. AND F. S. ROBSCHEIT-ROBBINS. J. Biol. Chem. **72:** 39, 1927.
STADIE, W. C. AND K. A. MARTIN. J. Clin. Investigation **2:** 77, 1925–1926.
STEARNS, W. H., C. K. DRINKER AND T. J. SHAUGHNESSY. Am. Heart. J. **15:** 434, 1938.
SUDHUES, M. Arch. f. Hyg. **109:** 135, 1932.
THEORELL, H. Biochem. Ztschr. **268:** 64, 1934.

TSCHERKESS, A. AND W. MELNIKOVA. Arbeiten u Materielen d. ukrainischen Staatsinstituts f. Arbeitspath. u Arbeitshyg. Charkow, 1928.
VEALE, A. P. Trans. Instn. Min. Eng. **63:** 417, 1922.
WALTERS, F. M. Am. J. Physiol. **80:** 140, 1927.
WARBURG, O. Biochem. Ztschr. **177:** 471, 1926.
WHIPPLE, G. H. Am. J. Physiol. **76:** 693, 1926a.
Am. J. Physiol. **76:** 708, 1926b.
WILLIAMS, J. W. Obstetrics, p. 762, 1930.
WILLIAMS, I. R. AND E. SMITH. Am. J. Physiol. **110:** 611, 1935.
YANT, W. P., J. CHORNYAK, H. H. SCHRENK, F. A. PATTY AND R. R. SAYERS. U. S. Public Health Service, Pub. Health Bull. no. 211, 1934.
ZONDEK, H. Deutsch. med. Wchnschr. **45:** 678, 1919.
Deutsch. med. Wchnschr. **46,** 235, 1920.

FLUCTUATIONS IN BODY IODINE

WILLIAM T. SALTER

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston

Since the discovery of iodine by Courtois, 1811, (28), two events stand out in our unfolding knowledge of iodine in relation to physiological processes. These are the discovery of iodine in the thyroid by Baumann, 1895, (9), and the isolation of crystalline thyroxine by Kendall, 1914 (84). Yet in themselves these discoveries contributed little to the understanding of biological mechanisms. They are supremely important because they serve as jumping-off points for painstaking researches by scores of other students who, bit by bit, have brought our knowledge to its present pitch.

The chief impediment to these studies has been a lack of suitable techniques and, in particular, of analytical methods. This continues to be true, largely because of the very low concentrations of iodine found in most biological systems. Indeed, the major difficulty in reviewing work on the physiology of iodine lies in the evaluation of technical procedures. This point is evident from a study of classical monographs on the subject, such as those of Kendall (85), von Fellenberg (55), Scharrer (141), Harington (69), Elmer (36), Leipert (94) and McClendon (115). To these sources the present article owes much.

In 1850 Rabourdin (132) published his method for determining iodine, and forthwith there began a series of investigations which can be traced through Chatin (19) and von Fellenberg (55) to the present day. Although Bubnow (15) identified the thyroid colloid as protein in nature, it remained for Baumann (9) and Hutchison (81) to study thyroglobulin as an *iodo*protein. The identification of 3,5 di-iodo-l-tyrosine by Wheeler and Jamieson (165) established a working hypothesis for the protein's constitution which led to its isolation by Harington and Randall (72) many years later (1929). Meantime, Harington and Barger (71) established that thyroxine also is a derivative of tyrosine. Finally, in the last decade it has become clear that the thyroid hormone

is stored in a characteristic colloid form, containing at least two iodo-amino-acids in peptide linkage (Harington and Salter, 73).

In recent years attention has turned to the measurement of the minute amounts of iodine in the blood and in various tissues. These methods are still crude, considering their objective, but they have been developed to the point of dividing this iodine into characteristic fractions, i.e., "organic" and "inorganic." Recent discussions of such technique have been given by Elmer (36) and by McClendon (115). Amounts as low as 0.5 gamma (0.000,000,5 gram) can be measured within an error of 0.1 gamma, an accuracy satisfactory enough for many physiological problems. These procedures, however, demand a considerable analytical skill. When several microchemists analyze the same sample of blood and report values for iodine ranging from 7 to 70 micrograms per cent (135), it is clear that technique is still a major problem.

Fortunately, despite these technical difficulties, new improved methods are appearing which yield consistent results in different laboratories; and the fantastically high values for blood and urinary iodine concentrations, so common a decade ago, are rapidly disappearing. Consequently, it is possible already to report much information which is of permanent physiological interest. Simultaneously, other data dealing with pharmacological observations and with clinical conditions have accumulated in even greater volume. Although the iatrochemist has much to teach the physiologist about iodine metabolism, such studies fall perforce outside our present limitations.

I. Iodine Stores in Body Tissues. Justus (83) decided that all cells contain iodine, and in recent years reliable quantitative evidence has been adduced in favor of his point of view. Nevertheless, the iodine content of the whole human adult is small; it varies between 20 and 50 mgm. (154), (36, p. 84). (This amount is equivalent to less than 10 minims of liquor iodi compositus, U. S. P.) Of this whole quantity, the muscles contain one-half, the skin one-tenth and the skeleton one-seventeenth.

The total iodine in the blood of normal, fasting individuals is probably less than 10 gamma per cent, and contributes less than one-tenth of the whole iodine in the body. The human thyroid normally holds at least one-fifth of the total iodine, even though its mass is only one-five hundredth of the whole body. Thus the thyroid iodine (40,000 gamma per cent) is a thousand times as concentrated as muscle iodine (30 gamma per cent). Lower values for all tissues may be encountered

when the iodine supply is very low, but the normal thyroid maintains its capacity for the preferential trapping of iodine even when the amount of ingested iodine is greatly reduced.

The next highest iodine concentrations are found in other glands of internal secretion (20). These include the anterior pituitary (36, p. 86) (37), epiphysis, ovary, adrenal cortex (47) (36, p. 85) and parathyroid. In general, the iodine concentration of these endocrine organs is some fourfold that of other body tissues, which contain only about 20 to 30 gamma per cent. The increased concentration in the endocrines largely disappears after thyroidectomy (154) (36, p. 87), whereas the iodine in skeletal muscle and other tissues remains relatively undisturbed. This fact raises the possibility that iodine in these endocrine glands is of thyroidal origin, a question to be studied further.

TYPES OF IODINE COMPOUND IN THE ORGANISM. At the present writing, tissue iodine usually is classified as *1*, inorganic iodide, and *2*, organically bound iodine, which may be *a*, like thyroxine, insoluble in dilute aqueous acid, or *b*, like diiodotyrosine, relatively soluble in dilute aqueous acid.

Iodide. It is clear that most body fluids contain inorganic iodide, the concentration of which (roughly, 2 gamma per cent) will be discussed later. Likewise, red cells are known to contain inorganic iodide. These concentrations, it will be noted, are rather low under ordinary physiological conditions. They may possibly be increased up to tenfold in hyperthyroidism, and certainly after the therapeutic administration of iodide up to an hundredfold (see below, section II, p. 358).

Pincussen and Roman (131) studied the electrodialyzable iodine in the mixed tissues of white mice. The average iodine was 3.4 mgm. per cent, of which the ratio of organic to presumably ionized iodine was 1.85. Such figures for iodide represent merely the total mass of tissue, and neglect the possibility that some of the diffusible iodine might not be iodide; they represent, therefore, maximal possible values. In the thyroid itself, relatively little inorganic iodide is found, even after recent administration of iodide to the organism (69), when it may reach to 10 per cent of the total thyroidal iodine, only to disappear rapidly after the medication is assimilated.

Naturally Occurring Organic Iodine Compounds. The other chemical compounds which contain iodine are iodinated tyrosine and iodinated thyronine. When elementary iodine is fed, as in liquor iodi compositus, U. S. P., it soon is changed in the gastrointestinal tract either into organic iodide or, by combination with protein split-products, into

diiodotyrosine. Likewise, the iodine-poor human thyroid fixes circulating inorganic iodide in the form of diiodotyrosine soon after its administration (65). Recently Hertz, Roberts, Means and Evans (78) have studied the fixation of radioactive iodine and have found that the process may be nearly complete within 15 minutes after a single dose, given intravenously. Subsequently, iodine is found in the form of tetraiodothyronine (i.e., thyroxine). Whether the intermediary stage, diiodothyronine, is present in the thyroid gland is still unknown.

Studies of artificially iodinated proteins (11) (7) (108) suggest that other iodine compounds should be looked for in mammalian tissues. Indeed, monoiodotyrosine has been described (126) in hydrolysates of iodinated casein. Likewise, Bauer and Strauss (7) found evidence of iodohistidine (both the mono- and diiodo compounds). From the standpoint of chemical evolution, it should be noted that diiodotyrosine ("iodogorgonic acid") occurs in sponges and in "corals" (77). The origin of thyroxine in the evolutionary scale still remains to be studied carefully.

The Thyroid Reserve. The thyroid protein itself, iodothyroglobulin, is a composite molecule containing both diiodotyrosine and thyroxine in peptide combination as integral parts of the molecule (73). In laboratory preparations the size of its molecular aggregate has been determined by ultracentrifugation to be about 670,000 (75). Under special laboratory conditions, however, this aggregate may split into four particles (156), and there is no certainty as to its size in vivo. It seems highly probable, however, that its colloidal properties serve to anchor the hormone in its storage form, the "thyroid colloid" of the microscopist.

A detailed study of the composition of thyroglobulin, with especial reference to its component amino-acids, has been made recently by Cavett (18) and by Brand and Kassell (14). It is by no means clear yet that the protein is a chemical entity in the sense that the crystalbumin of blood plasma is a definite entity. The protein has never been crystallized. Furthermore, depending upon the supply of iodine available, the iodine content of thyroid protein may vary from 0 to 1 per cent (8). The relative proportion of thyroxine to diiodotyrosine also varies. This ratio tends to be low when the total iodine is low (102), as described in another section. In thyroglobulin prepared from the glands of Argentine sheep by the author, for example, the partially purified, heat-coagulated protein contained over 1.2 per cent iodine, of which over 60 per cent was apparent thyroxine. By contrast, average human thyroglobulin in Boston contained 0.22 per cent iodine, of which

only 25 per cent was thyroxine-like. Nevertheless, Brand and Kassell have demonstrated a systematic intramolecular arrangement of those amino-acids which do not contain iodine.

Although it has been suggested (63) that thyroxine is lightly bound to a colloid vehicle, it seems clear that l-thyroxine is liberated from firm union in the peptide chain by the action of proteolytic enzymes (73). In normal thyroglobulin, both the diiodotyrosine and the thyroxine are in the l-configuration (72) (73). It remains to be seen whether the protein of thyroid cancer contains the d-forms, as suggested by the work of Kögl and Erxleben (90). The latest evidence seems *not* to favor this possibility.

The Thyroid Colloid. By microdissection methods fresh thyroid follicles may be "teased" apart and from them the intrafollicular colloid may be withdrawn as a viscid, albuminous liquid (134). The crude colloid in the follicle is adulterated with varying amounts of nucleoprotein (74). This intrafollicular nucleoprotein, however, has no known physiological function and is thought to originate from disintegrating or secreting cells of the follicular wall. It will not be considered further in this review.[1]

It is not known definitely in what chemical form the hormone is released from the colloid stored in the follicle. By enzymic hydrolysis Harington and Salter (73) obtained a peptide preparation of thyroxine which was assayed in human myxedema by Salter, Lerman and Means (138). Its greater solubility suggested that it might resemble the true circulating hormone. Further assays suggested that the remaining non-thyroxine iodine in thyroid protein also contributed to its potency in human myxedema. Harington (70) summarized this paradox by suggesting that the active thyroid secretion consisted of a peptide chain containing both thyroxine and other amino-acids, including diiodotyrosine. This problem is interesting from a therapeutic standpoint and will be discussed further in section III.

Other Tissues. Not much is known about the state of the organically bound iodine in other tissues. There is a thyroxine-like fraction, "T," in the blood (43), and probably also a diiodotyrosine-like fraction, "D" (161). Neither of these fractions is supported by a positive chemical identification, although the "T" fraction has been confirmed by bio-assay (168).

Direct attempts to isolate thyroxine from tissues at the present time

[1] In addition, Williamson, Pearse and Cunnington (166) have adduced evidence of an inactive thyroid "secretion," nearly free of iodine, found in tissue showing "hyperplasia."

are complicated not merely by difficulties in technical manipulation, but also by a more fundamental and, at present, surprising difficulty. This complication may be summarized as follows:

In recent years it has been found that such a nondescript protein as casein, when iodinated and subjected to alkaline hydrolysis, yields compounds related to thyroxine (1) (108) (136). Indeed, even unhydrolyzed serum albumin, after iodination, relieves human myxedema in classical fashion (103). The method of isolation involved in treating these nondescript proteins is quite similar to that for isolating thyroxine from thyroid protein. Consequently, the recovery of thyroxine from any tissue might be merely the result of an artefact. An alternative interpretation would be that organically bound iodine in the body is always in thyroxine-like form, a supposition which is by no means suggested by the classical studies of artificial iodoproteins (150) (7).

Biological considerations, however, indicate obviously that some thyroxine derivative circulates from the thyroid to the tissues by way of the blood stream. Moreover, hormonal activity has been demonstrated in tissue. Thus Kommerell (91) fed muscle meat obtained from both normal and myxedematous animals. The latter flesh failed to increase oxygen consumption in test animals, although the normal flesh did so. Likewise, tissue extracts have been studied from the standpoint of their effect on metamorphosis. Also Zawadowsky and Perlmutter (170) have used the metamorphosis of axolotl to amblystoma to detect the presence of thyroid hormone. This biological phenomenon occurs completely with as small a dose of thyroxine as 10 gamma per 10 grams of body weight. Accordingly, when iodine concentration is rigidly controlled, the confusing effects of other iodine compounds can often be excluded. When this test is performed carefully, therefore, it is more specific than the Gudernatsch (64) tadpole method or Uhlenhuth's procedure (163), and offers greater hope for detecting thyroidal activity in tissue extracts or in body fluids. By this method, for example, Zawadowsky and Asimoff (169) were able to demonstrate increased hormonal activity of the liver, kidney and blood serum in guinea pigs which had received thyroid preparation about six hours previously.

Unfortunately, such attempts to estimate concentrations of hormone are at present in the pioneer stage. Obviously, improved micromethods for detecting thyroid hormone in blood and other tissues are badly needed.

TEMPORARY IODINE STORAGE. When iodine is administered from an

external source, there results a temporary stage of metabolic flux which alters the characteristic concentrations just described. These alterations vary with the source of extra iodine.

Iodide. The fate of inorganic iodide after absorption depends in part on the dosage used and on the physiological state of the recipient. Maurer and Ducrue (114) gave rabbits 10 gamma iodine per kilogram in this form and analyzed various tissues both one day and four days later. They found a great increase in the thyroid iodine, corroborating the classical findings of Marine (111) in the iodine-hungry dog. The liver, skin and lungs showed very high iodine contents as temporary storage depots, but after four days only the heart, kidneys and lungs remained unusually rich in iodine.[2] Increased concentrations have been found temporarily also in the stomach and parotid (104). Obviously, the organism does not retain extra iodide long. With larger doses in guinea pigs, the thyroid stores only 4 per cent of the extra iodine accumulated, but the skin, hair, muscles and bones store iodine for many days at a high concentration (55). The lungs and trachea show very high concentrations temporarily, but soon lose the extra iodine despite the fact that the expired air shows no increase in iodine content (114).

Organic Iodine Compounds. Simple organic iodine compounds likewise accumulate in the skin (106). Furthermore, after three weeks' daily administration, iodotryptoflavin is stored especially in the brain and kidneys (54). Experiments with diiodotyrosine are unsatisfactory because as yet there is no micromethod available for determination of traces of this compound.

The fate of thyroxine derivatives, however, has been studied both by iodine analysis and by the bio-assay method of Zawadowsky and Asimoff (169). Asimoff, Estrin and Miletzkaja (3) studied the effect of a single dose of "thyroidin" by mouth in dogs and roosters. They found that iodine accumulated in the thyroid, kidneys, liver, blood, pancreas, skin, heart and spleen. Likewise, increased iodine is found in the muscles and bones of the rat five days after a single intravenous injection of thyroxine (93). Small amounts of this iodine remain stored for a long time in the animals' tissues, especially in the liver, muscles, thyroid and skin (125). In another section the selective accumulation of thyroxine iodine by the hypophysis has been discussed (155). Whether the tuber cinereum shows this in even more marked degree (148) has not yet been decided.

[2] The distribution of this iodide throughout body fluids has been studied by Wallace and Brodie (145).

The biological method of Zawadowsky has yielded interesting information as to the fate of thyroxine derivatives in bodily economy. Zawadowsky and Asimoff (169) fed "thyroidin" to mammals and birds and estimated its concentration in various tissues by bio-assay. During the first few hours the hormone is trapped in the liver to a large extent. Thereafter it increases in the blood plasma and in the kidneys. After 24 hours the hormone largely disappears. Birds appear to destroy the hormone less rapidly than mammals.

II. The Circulating Iodine. Iodine in respired air. The atmosphere of lowlands near the sea may contain 0.4 gamma iodine per liter, so that a man conceivably might inhale from 3 to 5 gamma daily. Consequently, it is difficult to estimate iodine truly excreted by respiration. By actual measurement a man can exhale 10 gamma in 24 hours, i.e., 25 per cent of the total excreted (143). Presumably this iodine is largely in the form of iodide. Szász (157) found as much as 24 to 33 gamma exhaled per day in a mountain atmosphere which was essentially iodine-free, but this larger amount may be related to the large water excretion by the lungs at high atmospheres.

Iodine in cerebrospinal fluid and in lymph. The nature of the iodine in cerebrospinal fluid is still unsettled. Its concentration is lower than that of whole blood. The following pairs of comparative values are given in gamma per cent for blood and spinal fluid, respectively: 10.6 and 7.4 (68); 13 and 10 (80); 7.7 and 2 (117). It is likely that the cerebrospinal fluid contains no thyroid hormone (117), but further study is needed.

The lymph contains an appreciable quantity of iodine (149), but the mechanism which governs its concentration remains obscure. The puzzling fact is that dogs whose *blood* iodine is from 10 to 18 gamma per cent yield thoracic duct *lymph* which contains 42 gamma per cent. Furthermore, when the animals are fed plenty of milk, values of from 290 to 378 gamma per cent are found. Together, these findings suggest that the high concentration in the thoracic duct is due to contributions of iodine from the intestine and the liver.

Iodine in milk. In the normal lactating animal, considerable quantities of iodine are secreted by the breast (27). In the first day of the human puerperium the colostrum yields only 2 to 5 gamma iodine in 24 hours, although the iodine concentration ranges from 8 to 45 gamma per cent (36, p. 105). When lactation is established, from 20 to 47 gamma iodine may appear in 24 hours in a concentration of 3 to 12 gamma per cent (95). Thus the monthly excretion of iodine amounts

to from 2.5 to 3.2 mgm. (162). The chemical status of this iodine is not clear. The major part of milk iodine is attached to organic material either by chemical combination or possibly by adsorption (55). Studies of lactating cattle have shown that iodine in the milk varies with the season and with the type of fodder. Seacoast pasturage increases the milk iodine (142). There is much less iodine in butter fat than in skimmed milk (116). The nursing infant does not receive thyroid hormone by way of the milk (35), nor does the milk yield a thyroxine-like fraction after hydrolysis (45) (44).

IODINE IN SWEAT AND SALIVA. Iodine is excreted by the skin in variable amounts; data are not available on variations in concentration. In profuse sweating the total in sweat may be over 30 per cent of the total iodine excreted (55). On the other hand, Szász (157) found practically no cutaneous excretion in the mountains at 1000 meters' elevation.

The concentration of iodine in saliva may vary from 0 to 362 gamma per cent (149), but the total amounts excreted by this channel are small. After the therapeutic administration of iodine, high concentrations appear in the saliva within a few minutes (32).

BLOOD IODINE. *Physiological Range.* It has been clear, at least since the work of Kendall and Richardson (87), that normal blood contains iodine in a characteristic concentration. Normally, this amount is rarely less than 3 gamma per cent or more than 20 gamma per cent, as recorded by the majority of methods now available (36, p. 91). The blood of various animals is similar to that of man. Of course, the concentration is subject to fluctuation. Thus the level is increased somewhat by an iodine-rich diet; indeed, therapeutic doses of iodides (e.g., 1 gram of sodium iodide) may raise the level temporarily to several hundred gamma per cent. Likewise, a diet of iodine-rich foods, especially marine fish and seaweeds, increases the blood level. Sturm and Buchholz (154), among others, have found that seasonal variations in blood iodine occur parallel with changes in thyroid iodine. Löhr (107) failed to confirm this, but it should be remembered that in certain localities (e.g., the British Isles) thyroid iodine may not be subject to remarkable fluctuation (113). The blood iodine may rise at the time of the climacteric in women (16), but this phenomenon is not constant.

There is considerable diversity of opinion about the distribution of iodine between red cells and plasma. It seems clear that the plasma contains the major portion. Representative figures indicate about 8

to 12 gamma per cent in the serum and only 4 to 6 gamma per cent in the red cells (62). Trevorrow (161) studied a series of analyses of whole blood and plasma and found the total iodine to be distributed in proportion to the water content of the plasma and cells. This statement should be considered as a first approximation.

QUALITATIVE NATURE OF BLOOD IODINE COMPOUNDS. Ironically enough, despite many quantitative studies of the partition of the blood iodine, the nature of these fractions is largely unknown. Various methods have been used to fractionate the blood, but they are so arbitrary that it is difficult to compare results obtained by one method with those by another procedure. A systematic study of the blood iodine by rigorous physicochemical methods is sorely needed. Meanwhile, it is possible to draw tentative conclusions only.

Even if the plasma and red cells contain iodine in equivalent concentration in terms of water (161), there is no evidence that these concentrations necessarily represent the same chemical compounds. Indeed, Zawadowsky and Asimoff (169) found that, on feeding thyroid, the plasma became biologically active, but NOT so the red cells. It will be important to learn whether red cells contain hormone because, if not, the plasma should be analyzed separately, in view of its greater physiological significance.

There are many "normal fasting" values for "inorganic" iodide in the literature, but it is dubious whether much of the normal blood iodine is truly inorganic unless iodide has been ingested recently (161). Ultrafiltrates of plasma from fasting animals contain little iodine unless potassium iodide has been fed to the animal recently. On the other hand, iodide added to plasma can be recovered quantitatively in the ultrafiltrate. When animals are fed iodides, however, the extra inorganic iodide is distributed between plasma and cells in (roughly) equivalent concentration, i.e., in terms of the respective water contents of the two phases (114). Although these filtration experiments indicate that most of the normal blood iodine is bound by protein, Trevorrow (161) has found that it can be removed from the protein almost completely by extraction with butyl alcohol or by continuous extraction with ethyl alcohol or acetone. In other words, the union with protein may be due to adsorption rather than to firm chemical combination. Thus by varying the procedure, the normal fasting blood iodine can either *a*, be left almost entirely adherent to protein, or *b*, be removed almost completely from protein. Unfortunately, many of the procedures employed in the past 15 years represent intermediate stages.

Thus the classical procedure of Lunde, Closs and Pedersen (110) for the determination of "organische" iodine gave the fraction which happened to be bound to protein after *one* extraction with alcohol. Similarly, McClendon (115) recently has used acetone to find the "hormone iodine" left bound to protein, despite the finding by Eufinger and Schulte (52) that all of the iodine can be removed by acetone if further extraction be made. In other words, it is hazardous to assume that alcohol-soluble (or acetone-soluble) iodine represents "inorganic" iodide, whereas the alcohol-insoluble (or acetone-insoluble) represents "organic" or "hormone" iodine alone. Similarly, the use of dialysis or electrodialysis (10) gives values for diffusible "iodide" which depend upon changing concentrations and rates of diffusion.

Recent analytical studies (161) (127) indicate that thyroxine, diiodotyrosine, thyroxine-proteose and thyroglobulin are all nearly completely precipitated with the proteins of blood by such agents as heat and acetic acid or zinc sulfate and sodium hydroxide. On the other hand, added iodide escapes precipitation. Such observations suggest that the normal fasting blood iodide concentration is only about 2 gamma per cent. Contrast this figure with the high values for alcohol-soluble "iodide" now in the literature! The author suggests that non-precipitable iodine be regarded henceforth as an index of inorganic iodide.

The protein-bound iodine of the plasma also may prove to be a useful measurement provided care is taken to obtain the maximal value possible. This figure would include the various constituents of thyroglobulin mentioned above, if present, and possibly other unknown iodine-containing substances which can be adsorbed on protein molecules. Indeed, when thyroxine is added to blood it may appear either in the "inorganic" or the "organic" fraction, depending upon the procedure employed (128). This fact may explain why Leipert (96) was able to fractionate diffusible iodine into fractions precipitable by silver and not so precipitable. Lipoid-bound iodine also has been suggested (164), but it seems insignificant if present at all (96).

Trevorrow (161) has been able to separate the butyl alcohol extract of blood iodine into thyroxine-like fractions and diiodotyrosine-like fractions. She concludes that "the greater part of the blood iodine possesses properties similar to those of thyroxine and diiodotyrosine, and a portion of this is not diiodotyrosine but is thyroxine-like in its solubility."

In this connection, immunological observations recently made by Lerman are illuminating. He produced rabbit antiserum "which would

detect human thyroglobulin in concentrations as low as 0.15 to 0.3 mgm. per cent, i.e., in a concentration equivalent to less than 1 microgram per cent of iodine. *This was true whether the thyroid protein was dissolved in physiological salt solution or in normal human serum.* He was unable to detect such a concentration in normal human serum, thyrotoxic serum (pre- or postoperatively), or in myxedema. An exception to this statement was the finding that after surgical manipulation of the thyroid, hormone can be detected by this means frequently in the thyroid veins during operation and occasionally in the systemic venous blood for as long as 24 hours after operation. He was unable to detect any thyroglobulin in human urine. One preparation of the antiserum, which was unusually potent, was capable of disclosing a concentration of thyroglobulin as low as 0.05 mgm. per cent. With this potent antiserum Lerman was unable to detect any thyroglobulin in serum from hyperthyroid patients (98). These observations suggest strongly that the circulating hormone differs from the storage form previously described in section I.

For the sake of uniformity in the literature, the author suggests that in clinical and metabolic studies the following program be followed: *1*. Plasma to be separated from red cells, at least until it appears that the iodine of red cells reflects precisely that of the plasma. *2*. The plasma iodine to be separated into "I" iodine, presumably iodide because ultrafilterable or non-precipitable, and into "P" iodine, the maximum adsorbable on protein or precipitable with protein molecules. *3*. The "P" iodine to be separated, according to Leland and Foster (97), into a "T," thyroxine-like fraction and "D," diiodotyrosine-like fraction. This plan will admit of direct comparison of concentrations in the blood with those in the thyroid gland itself, or with pharmaceutical preparations and derivatives of thyroglobulin or of iodoprotein (102) (103). Indeed, in one such experiment with beef plasma, Trevorrow found 92 per cent of the total iodine in the "P" fraction, of which one-third was "T" iodine and two-thirds "D" iodine. These are the values one would have expected from the normal human thyroid in Boston!

After human blood has been hydrolyzed directly in alkali, it yields both "T" and "D" fractions (43). Although the hydrolysis admittedly destroys some 20 per cent of added thyroxine, nevertheless it increases qualitatively the reliability of the results. The "T" iodine is between 2.9 and 4.8 gamma per cent, i.e., about one-third of the average total iodine (12 gamma per cent). Thus 5 liters of human blood would

contain nearly 0.5 mgm. of apparent thyroxine; 25 liters of horse blood, 1 mgm.; and the entire blood of a dog 0.05 to 0.025 mgm. of apparent thyroxine.

Extant Clinical Data. These academic considerations notwithstanding, there exists a large mass of clinical and metabolic material based on the concept of "anorganische" and "organische" iodine as separated by the alcohol precipitation of Lunde and Closs (109) or by similar procedures. Because these data convey important information, we must review them carefully, remembering that "anorganische" iodine often contains little iodide and that "organische" iodine represents only a portion of the maximal iodine which is adsorbable on protein. The "organische" iodine is sometimes called "hormone iodine" (115). The consistent behavior of this value in normal and in hyperthyroid individuals offers some hope that eventually this arbitrary fraction may be closely identified with the "T" fraction described above, even though it probably constitutes the net effect of several compensatory errors.

Fasting Blood Iodine. Because of the technical difficulties encountered in separating small amounts of iodine, not to mention the diversity of analytical techniques in vogue at present, comparative values obtained by the same investigators are more reliable than absolute values obtained by different procedures in various localities. Obviously, when stating blood iodine in studies of pathological physiology, each investigator should make clear his range for normal values.

As regards whole blood iodine, fasting values obtained by Gutzeit and Parade (67) (67) in Breslau are instructive:

For normal men: 9 to 16 gamma per cent (average 14 gamma per cent)
For normal women: 11 to 20 gamma per cent (average 17 gamma per cent)
"Organische" iodine: 3 to 5 gamma per cent

$$\text{Normal "iodine quotient"} = \frac{\text{"organische"}}{\text{"anorganische"}}, \text{ 0.2 to 0.5}$$

In Boston, Perkin, Lahey and Cattell (129) found in normal adults an average of 6.6 gamma per cent, ranging from 1 to 10 gamma per cent. In Ohio, Davis, Curtis and Cole (33) found in normal adults an average of 10.1 gamma per cent, ranging from 8.5 to 16.2 gamma per cent. In children in New York City, Fashena (53) found average values, without sex difference: for infants less than 24 hours old 4.7 $\pm$ 0.33 gamma per cent, ranging from 1.0 to 11.0 gamma per cent; for others up to 13 years, 6.6 $\pm$ 0.15 gamma per cent, ranging from 3.0 to 12.0 gamma per cent.

Effect of Exercise. In studying blood iodine, it must be remembered that vigorous muscular activity causes an increase in the fasting blood iodine within a few minutes. There is disagreement about the extent to which the iodine rises; McCullagh and McCullagh (118) report a rise of about 9 gamma per cent, whereas Herxheimer, Mislowitzer and Stanoyéwitch (79) found increases of over 150 gamma per cent. The normal level is not restored for over two hours after cessation of the work.

Circulating Iodine after Administration of Iodine Compounds. The total blood iodine concentration at a given moment represents the net result of many complicated processes. Among these are the quantity and type of compound used, the rate of intestinal peristalsis (i.e., presence of diarrhea), the ease and rate of intestinal absorption or of parenteral injection, the avidity of the thyroid for new iodine, the permeability of various tissue cells, the state of kidney function and many other factors. Furthermore, different species of animal react differently. Consequently, manifold variations are possible and it is possible to consider only certain typical combinations of circumstances.

Iodide Administration. Potassium iodide is so rapidly absorbed that the blood iodine concentration rises to a peak within 30 minutes if doses of 2 grams or more are ingested (154). Under these circumstances, the blood iodine concentration may rise well above 1000 gamma per cent. With smaller doses (e.g., 0.5 gram) the peak is not reached until 90 minutes (164). Then the blood iodine falls, to reach the initial level in 48 hours. Thus the bulk of the iodine appears rapidly in the circulation and again disappears rapidly (38). Elsewhere (171), iodine tolerance curves are discussed to show that the functional activity of the thyroid regulates this fluctuation in blood iodine to a large extent. Eventually a large part of the extra iodine is eliminated through the kidney. A much smaller amount is eliminated into the intestine through the bile and intestinal secretions. Possibly the stomach also eliminates important amounts of iodine (104), thus adding to the intestinal content.

After intravenous injections of iodine, the blood level drops rapidly from its initial peak. The type of curve obtained, however, is determined largely by the dose injected. Elmer (36, p. 155) has injected small doses (1.7 mgm. potassium iodide) and found that in normal individuals the blood iodine returns to control levels in six hours. Also Perkin and Lahey (130), using larger doses (i.e., 0.6 gram by mouth), found a more protracted hyperiodemia, as did Fitz (57).

Even though the total blood iodine rises, the "*organische*" moiety thereof falls (164). Thus, after the oral administration of 0.5 gram of potassium iodide the "organische" moiety may drop from 10 gamma per cent to 3 gamma per cent when the total iodine approaches its peak. Later, the total iodine approaches the normal after 48 hours, as does the "organische" moiety. Thus the curves for total and "organische" iodine, respectively, at first separate and then approach each other again. It may be that the reason for the drop in "organische" iodine is suppression of the thyroid secretion (152) (110). If true, the rapidity of the fall in concentration is remarkable because thyroid changes are usually considered sluggish.

Administration of Thyroxine Derivatives. The blood iodine curve following the administration of whole thyroid or of thyroglobulin has not been studied well. Veil and Sturm (164), however, studied the effect of feeding small doses of a desiccated preparation called "thyroidin" (containing 150 gamma iodine). In two hours the peak was reached at a questionable 41 gamma per cent, and in 24 hours the blood was normal. More studies are needed on this point. Both "organische" and "anorganische" fractions increase, suggesting that decomposition of the hormone occurs in the gastrointestinal tract, in the liver (49), or elsewhere. Nevertheless, after the enteral administration of thyroglobulin some antigen (presumably thyroid protein) is found in the blood stream (76). Most of the ingested thyroglobulin is split, however, as expected, into peptones or peptides in the gastrointestinal tract (6) (5).

Thyroxine per Os. When thyroxine is administered by mouth appropriately dissolved in alkali,[3] the blood iodine of dogs reaches 28 gamma per cent in two hours and falls to normal in 12 hours (146). After injecting 2 mgm. of thyroxine, Böe and Elmer (12) found that there was an almost immediate fall from the initial peak of 36 gamma per cent. In 24 hours the blood was again normal. It is highly significant that the rise was entirely in the "organische" moiety.

Relation of Blood Iodine to Basal Metabolic Rate. It is generally stated that the blood iodine level does not run closely parallel to the severity of the disease. Nevertheless, Möbius and Nolte (124) found a certain correlation between the increase in basal metabolic rate and the increase in blood-iodine concentration. The author suggests that the correlation might be greater if the logarithm of the plasma con-

[3] Thompson and his collaborators (159) have shown that about 80 per cent of the drug is assimilated, when administered in this fashion.

centration be studied. He has compiled in such fashion the data of Elmer and Scheps (50), who stated that they failed to find an exact parallelism. These data show a considerable fidelity to the equation:

$$\text{B. M. R.} = \log (\text{Blood iodine minus } 3)$$

when blood iodine is expressed as micrograms per 100 cc. This relationship is shown in figure 1. The coefficient of correlation $r = 0.75 \pm 0.07$ and P is less than 0.01; i.e., the chances that this correlation is significant are better than 100 to 1.

When the "organische" moiety of the blood iodine is investigated, it is generally found to be increased more consistently than the total iodine (164). Gutzeit and Parade (66) have tabulated the ratio of "organische" to "anorganische" iodine. The normal quotient is 0.2 to 0.5, whereas in severe hyperthyroidism it may approach 4.0. Sometimes, however, the basal metabolic rate is found to be markedly increased with a fairly low "organische" iodine fraction (39). Likewise, the inverse combination may occur (34). Various factors conspire to obscure this relationship, viz., inaccuracy of present analytical methods, the possible presence of inactive organic iodine compounds (possibly degradation products of the hormone) and the difficulty of attaining in these patients a "steady state," physiologically speaking.

Gutzeit and Parade (67) (66) have laid great stress upon the behavior of the "organische" iodine and of their "iodine quotient," i.e., "organische"/"anorganische" fraction. In severe hyperthyroidism, these measurements indicate that the gland is working at maximal capacity, as indicated by the response to exercise. In normal persons, within an hour or two after vigorous exercise the "organische" iodine rises, and likewise the iodine quotient, to return to normal after 24 hours. In hyperthyroidism, on the contrary, there is no further rise and there may even be a fall in these values.

Elmer, Rychlik and Scheps (46) attempted to simplify the problem by determining the level of "thyroxine-like" iodine substances in the blood after hydrolysis. They used the method of Leland and Foster, slightly modified. In three cases studied the blood contained from 9 to 16 gamma per cent of "thyroxine" iodine, i.e., 50 to 60 per cent of the "organische" iodine or 27 to 44 per cent of the total iodine. Apparently a thyrotoxic man has from 0.8 to 1.4 mgm. of thyroxine in his blood, as compared with 0.4 to 0.5 mgm. in the normal person. More recently, Trevorrow (161) has improved the analytical technique so that, as data continue to accumulate, it may be possible to demonstrate a closer

parallelism between the basal metabolic rate and the concentration of presumable hormone or some mathematical function of it.

III. Thyroid Activity. Although the thyroid is the chief regulator of iodine metabolism, it is not the purpose of this review to discuss the

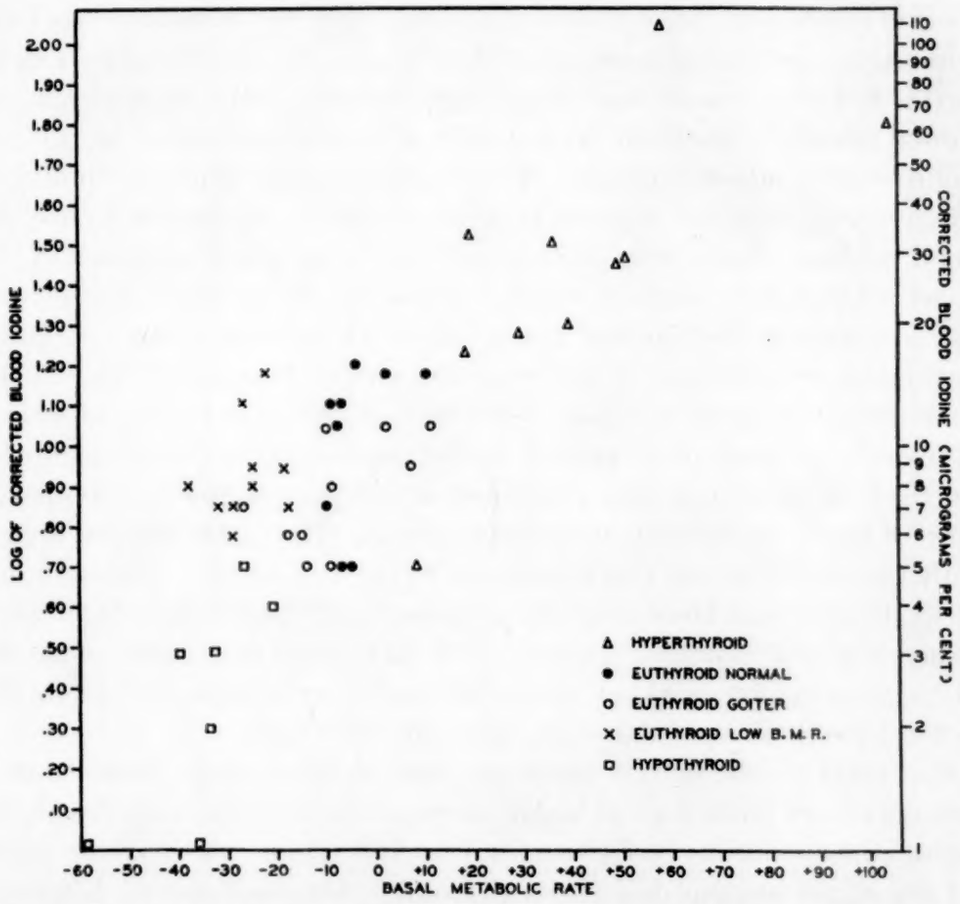

Fig. 1. Blood iodine and basal metabolism. Data for blood iodine concentration in several states of thyroid function, collected by Elmer and Scheps in Lwów. An arbitrary correction of 3 gamma per cent for inorganic iodide has been subtracted from the total blood iodine. The scale showing iodine concentration is logarithmic. The high correlation with basal metabolic rate leads to the inference that the two are related if 1, clinical classification is adequate; 2, analytical technique good; 3, exogenous iodide intake is low, and 4, a steady physiological state is attained before analysis is made.

thyroid gland, its chemistry and physiology (69). It must suffice here to consider certain quantitative aspects of its endocrine product, especially as it concerns iodine compounds and their calorigenic effect.

Manufacture of hormone. When elementary iodine or iodates

are fed, they are converted into iodide before absorption (22). Both iodine and diiodotyrosine can be absorbed from the intestine into the blood, and both can be removed from solutions perfusing the isolated thyroid gland. Although the gland may receive iodine from tincture of iodine painted on the skin or buccal mucosa (160) or by inhalation of ethyl iodide (99), nevertheless the usual source for hormonal synthesis evidently is iodide in food or medication. Using radioactive iodide, Hertz, Roberts, Means and Evans (78) recently have shown that the normal gland is surfeited with newly administered iodine within 15 minutes after administration. The newly trapped iodine is combined progressively with the thyroid protein, or else it diffuses back into the blood, so that after a day or two little inorganic iodide remains in the gland. Ultimately, only a small fraction of an ordinary therapeutic dose remains in the normal gland, although an iodine-starved gland may retain as much as 18 per cent of a single therapeutic dose (111). With repeated doses the iodine content of the gland may be raised to over 1 per cent, dry weight. After such treatment, for example, the thyroids of guinea pigs contained 57 mgm. of iodine, as compared with 15 mgm. in thyroids of control animals (114). Of course, organically bound iodine can also contribute to thyroid stores. For example, Klein, Pfeiffer and Hermann (89) administered "jodtropon" for a week to hemithyroidectomized animals. Of 22.6 mgm. of iodine so given, 5.1 mgm. were retained, of which 1.9 mgm. were recovered from the single thyroid lobe and 3.2 mgm. from all other tissues.

The total iodine in the whole thyroid of apparently normal adult men may vary from 2 to 28 mgm. of iodine (112). In fresh tissue the iodine concentration varies from 1.1 to 166 mgm. per cent (or 12 to 431 mgm. per cent in desiccated thyroid). Marine believes, however, that human tissue outside of the limits 0.1 to 0.55 per cent (by *dry weight*) should be considered abnormal. The difficulty in assessing normal values is enhanced by the fact that the iodine content varies with diet, topography, season, endocrinological balance and age. Furthermore, if iodine intake is low, not only is the content of the thyroid low, but compensatory hyperplasia of the tissue further reduces the gross iodine concentration and exaggerates the deficiency. Of the iodine so stored, normally about one-third is in "thyroxine-like" form in human glands, although in certain animals (e.g., Argentine sheep) this value may approach two-thirds. In other words, the major portion of the iodine incorporated in normal human thyroid protein has reached

the diiodotyrosine stage, but has not been built up into the more complex thyroxine.[4] When the gland is secreting hormone at full capacity, its total iodine reserve may be depleted to less than one-tenth of the normal average. Furthermore, its thyroxine-like reserve may nearly disappear, presumably because it is removed as fast as it is synthesized.

Various estimates of apparent thyroxine in the whole human thyroid indicate consistently a store of from 3 to 5 mgm. (37), from 0.2 to 9 mgm. (97) or from 0.9 to 7 mgm. (65). In dog thyroids, Elmer (37) found 0.7 to 1.1 mgm. of apparent thyroxine, in rabbits only 3 to 5 gamma. The corresponding figures for diiodotyrosine are 2.0 to 6.1 mgm. iodine in human thyroids, 0.2 to 1.19 mgm. in dog thyroids and 4 to 18 gamma in rabbit thyroids. These latter figures include inorganic iodide, which is presumed to be negligible.

Ebb and Flow of Thyroid Iodine. The gland can regulate iodine metabolism either by fixing iodine and anchoring it to colloidal molecules, or by releasing such iodinated molecules or fragments thereof into the circulation. The action of the gland depends largely upon the properties of the blood which perfuses it. Foot, Baker and Carrel (58) have studied isolated human thyroids preserved in the Lindbergh apparatus, and found that the final histologic picture depended on the nature of the perfusate, *not* on the previous condition of the gland. Likewise, Sturm (152) was able to study the result of various concentration gradients on the storage of thyroid iodine by working with isolated thyroids perfused with solutions containing various concentrations of iodide. Up to 500 gamma per cent of iodide in the perfusing fluid, the gland removes iodide from the arterial blood, thus producing a demonstrably lower venous concentration. Above a concentration of 500 or 600 gamma per cent, a short period of iodide retention is followed within 20 minutes by iodine elimination, so that the iodine level in venous blood is higher than in the arterial blood. Thus the gland's iodine reserve is reduced and organically bound iodine is released. This latter situation seems highly abnormal, and probably has little direct physiological significance, but it does demonstrate that the storage mechanism is reversible. It is puzzling that organic preparations like "jodtropon" or even thyroxine, when perfused through the isolated gland, are not themselves stored. Nor do they influence retention or release of thyroid stores (152). Because this finding might

[4] It is conceivable that a very small amount (2 per cent) of the iodine may exist in lipoid combination (164).

suggest that iodine can enter the thyroid only in inorganic form, it deserves further study. Unfortunately, such data on diiodotyrosine are not available.

Sturm (152) also tested surviving thyroids from dogs which had been "satiated" with iodide for one or two weeks previously. These glands, when perfused with a solution containing iodide, quickly reacted by elimination of iodide! Three weeks after cessation of iodide feeding to the animals, the gland approached the normal reaction. Similar studies were made with glands from dogs which had been rendered thyrotoxic by administering "thyroidin" or thyroxine for several weeks. Such glands were atrophic, reduced to nearly half the normal size and showed flattened acinar epithelium lining follicles filled with colloid. Four such glands, however, reacted normally to perfusion with iodide by storing iodine. One other excreted iodine when the concentration in the perfusate reached 220 gamma per cent. Apparently the hormonal iodine, exogenous in origin, does not often yield enough iodide (by decomposition) to satiate the gland.

Daily Production of Hormone. On the basis of substitution therapy in human athyreosis, it seems clear that the human thyroid secretes about $\frac{1}{3}$ mgm. of thyroxine or its equivalent in 24 hours. On the basis of iodine balances, the optimal daily requirement is not less than 100 gamma iodine nor more than 200 gamma iodine (36, p. 277). Means (119) summarized his own data and the data of others, and reached much the same conclusion. Eppinger and Salter (51) confirmed his value by treating human myxedema with purified human thyroglobulin.

The amounts of hormonal iodine in the body can be estimated from the rate of decay of the metabolic rate in myxedema (13). Such calculations suggest that, exclusive of the thyroid, an average human adult contains some 10 to 20 mgm. of iodine, of which about two-thirds is in the form of thyroid hormone. These values are surprisingly consistent with actual analyses of tissues given elsewhere. The author and Lerman (100) have similarly observed the daily consumption of a single hyperthyroid individual at various levels of metabolism. This patient, previously reported by Means and Lerman (120), was subsequently treated alternately with crystalline racemic thyroxine (intravenously) and with Armour's desiccated thyroid (U.S.P.) by mouth. Taken together with *1*, the decay curve, and *2*, the iodine response reported by Means and Lerman (120), these additional data—i.e., on *3*, daily utilization at various levels; *4*, differential effect of thyroxine as compared with whole thyroid, and *5*, the theoretical hormone content of the

body tissues, as estimated by integration of the decay curve against time,—all collected on a single individual, are extremely interesting because they supplement and reinforce one another. Data on items

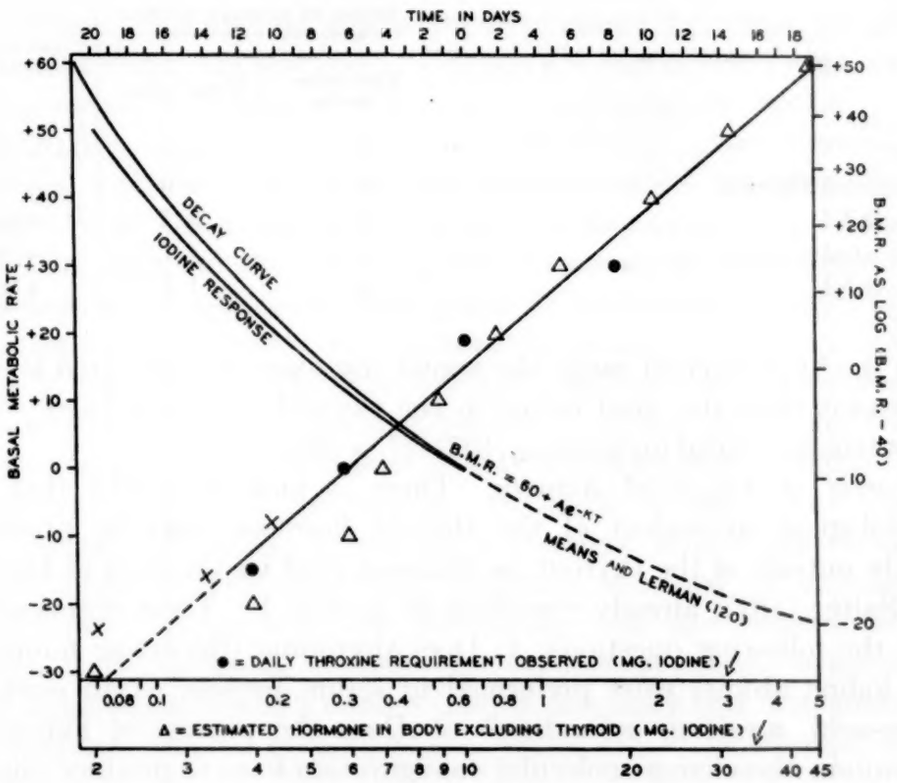
TIME IN DAYS
BASAL METABOLIC RATE
B.M.R. AS LOG (B.M.R. - 40)
DECAY CURVE
IODINE RESPONSE
B.M.R. = 60 + Ae^-kt
MEANS AND LERMAN (120)
● = DAILY THROXINE REQUIREMENT OBSERVED (MG. IODINE)
Δ = ESTIMATED HORMONE IN BODY EXCLUDING THYROID (MG. IODINE)

Fig. 2. Requirement of hormone with varying metabolism. Collateral data on a single individual, reported in part by Means and Lerman (120), whose curves for iodine response and metabolic decay are reproduced. The straight line serves three purposes, provided the appropriate scales are observed: *a*, with the right-hand ordinate and the topmost abscissa, the curve of Means and Lerman is illustrated as a logarithmic function; *b*, with the left-hand ordinate and the lowest abscissa (open triangles), the hormone stored in extrathyroidal tissue is shown, as estimated by integration of the decay curve against time; *c*, with the left-hand ordinate and the intermediate abscissa (solid circles) are shown actual observations by Lerman and Salter (100) of the requirement of thyroxine-iodine at several levels of hyperthyroidism. These data are supplemented by data (crosses) of Thompson (158) from another case recovering from myxedema.

1, *2*, *3* and *5* are graphically presented in figure 2. The daily utilization, *3*, and the calculated content of the organism, *5*, both rise logarithmically with the basal metabolic rate. The dosage (100) required at a steady state of metabolism is shown below, together with comparative

effects of whole thyroid and thyroxine. The desiccated thyroid, by analysis, contained 29 per cent of its iodine in the form of thyroxine.

Response to thyroxine and desiccated thyroid

MEDICATION	IODINE IN SUBSTANCE USED (MGM. PER DAILY DOSE)		LEVEL OF B.M.R.
	Thyroxine-iodine	Total iodine	
None	0	0	−15
Desiccated thyroid	0.17	0.6	0
$C_{15}H_{11}O_4NI_4$	0.5	0.5	+19
Desiccated thyroid	1.4	4.7	+30
$C_{15}H_{11}O_4NI_4$	4.7	4.7	+60

In the hyperthyroid range the actual thyroxine administered is more important than the total iodine in the thyroid substance used. This is not true in clinical myxedema (102) (cf. p. 368).

Genesis of Thyroidal Activity. There is now no doubt that the physiological equivalent of the thyroid hormone may be produced readily outside of the thyroid, as demonstrated by the data of Lerman and Salter (101), already described in section I. Their observations raise the following questions: *1.* Does thyronine (thyroxine minus all four iodine atoms) exist preformed in serum protein as an essential amino-acid, awaiting iodination? *2.* Does the process of iodinating the protein also change molecular configuration so as to produce physiological activity? *3.* Can the thyroidless organism synthesize iodothyronine molecules from other iodinated residues?

The classical, rapid recovery of these individuals confirms Abelin's report (2) of the production of extrathyroidal hormone. Moreover, the fact that serum albumin, when simply iodinated, can completely relieve human myxedema casts doubts upon the prevailing conception that the gland's function is specifically to manufacture a hormone de novo. But lately, the synthesis from diiodotyrosine has been conducted directly in vitro (17). In vivo, Condorelli (25) found that dogs treated with 0.1 gram of diiodotyrosine daily remained healthy despite thyroidectomy. On the other hand, only feeble calorigenic action can be demonstrated when huge doses of diiodotyrosine are administered to human patients with myxedema (158). There seems to be some peculiar function of protein combination, perhaps in orienting hydroxyl groups or in sealing off the amino-acid chains. Indeed, Cohn, Salter and Ferry (23) noted a strange disappearance of tyrosine acidity on titrating iodoglobin.

Recently, Ludwig and Mutzenbecher (126) have reported the isolation of thyroxine itself and of monoiodotyrosine from alkaline digests of iodinated casein and serum protein. Working with serum albumin, the author has obtained only a substance resembling diiodothyronine and a tetraiodo compound resembling deaminated thyroxine, but giving no Kendall-Osterberg reaction. This work is highly interesting, but as yet too recent to be evaluated from a physiological standpoint.

Ordinarily, however, it is clear that the thyroid gland supplies the organism with the form of thyroid hormone which circulates in the blood. What this naturally occurring form may be is still a matter for conjecture, although the following observations on the calorigenic effect of various iodine compounds have definitely limited the possibilities.

CALORIGENIC ACTIVITY OF THYROID HORMONE IN VARIOUS FORMS. There is little doubt that thyroxine (85) qualitatively reproduces the full effect of the thyroid hormone, because in human myxedema substitution therapy with crystalline thyroxine, judiciously administered, affords complete relief indefinitely. It should be emphasized further that the relief of human myxedema is the most reliable test available for thyroidal activity, and that results so obtained by experienced clinical investigators are more convincing than the measurement of oxygen consumption in animals or than metamorphosis experiments with tadpoles (163) and axolotl. This is especially true if quantitative observations lead to generalizations which are to be applied in the clinic.

Harington and Salter (73) isolated levorotatory thyroxine from thyroid protein. When the d- and l-forms made in Harington's laboratory were tested by Salter, Lerman and Means (139) in human myxedema, no difference in activity could be discerned. In tadpole metamorphosis and in rat metabolism, however, Gaddum (61) found l-thyroxine to be thrice as effective as the d-form. This material also came from Harington. Similarly, Foster, Palmer and Leland (59) found in normal guinea pigs that l-thyroxine is twice as active as dl-thyroxine; hence, they infer the d-form to be inactive, but no direct test of the d-form was made to prove it inert. The author believes that the difference in these results is due to biological dynamics, i.e., differences in rates of excretion, destruction and storage in various species receiving various dosages at different levels of thyroid function.

The Natural Hormone. There remain two puzzling facts with regard to thyroid activity which involve not only the true character of the natural hormone but also its pharmacological assay and dosage. The

first fact, pointed out by Kendall, is that certain samples of desiccated thyroid yield no crystalline thyroxine although they are highly potent physiologically. The second fact is that desiccated thyroid or fresh thyroglobulin may produce more calorigenic effect than does the thyroxine contained therein (73).

In view of these facts, both Kendall and Harington have suggested that thyroxine itself is not the true hormone in its natural form. Indeed, Barger (4) suggested that the thyroid contained a more active substance than thyroxine, i.e., a compound which breaks down during isolation or is converted into the less active thyroxine. Kendall and Simonsen (88) also speak of two hormonal forms: *1*, thyroxine, as we know it, and *2*, "active thyroxine." They suppose that the latter substance is the physiologically active molecule and suggest that it may be a hydroxy-thyroxine. Thus, in the tissues compound *1* becomes compound *2* as the first stage of pharmacological metabolism.

Harington (70) suggests that the true hormone contains both thyroxine and diiodotyrosine, in peptide combination, connected by a link or bridge of other amino-acids. Nevertheless, neither thyroxyl-diiodotyrosine nor diiodotyrosylthroxine, synthesized in Harington's laboratory, is as active as thyroxine itself.

Potency of Thyroid Preparations. The discrepancy between the calorigenic activity of thyroxine and whole thyroid deserves special comment, not only because it is important pharmacologically and clinically, but also because it is still the subject of dispute. Thus Foster, Palmer and Leland (59), working with normal guinea pigs, found only the thyroxine fraction to be effective. Likewise, Thompson, Thompson, Taylor, Nadler and Dickie (159) found that desiccated thyroid on the average yielded only 62 per cent as much activity as intravenous thyroxine. They obtained variable results with different samples of desiccated thyroid.

On the other hand, investigators in Boston (121) (102) (139) (119) have treated several scores of cases of human myxedema with thyroglobulin and its various derivatives and find that, in proportion to the iodine it contains, whole thyroid is distinctly more active in these patients than pure thyroxine. Similar results have been reached by Freud and Laquer (60), using a digestion product of thyroglobulin, "thyranon." More recently, Meyer (122) has confirmed these results in rats and studied the effect of thyroidectomy:

> Male rats were thyroidectomized at about 80 days of age and allowed to progress into hypothyroidism for six or eight weeks. Their oxygen consumption was

used as the criterion of the activity of thyroid medication, the dose of which was adjusted to produce an average increase of 30 per cent in metabolic rate. The normal rat proved to be too variable to serve as a reliable test object. Moreover, thyroidectomized animals were found to be about 25 times as sensitive to a given dose of thyroxine as the normal animal, because the former group required only 0.75 gamma of thyroxine to produce the standard rise in metabolism of 30 per cent. The data lead to the following conclusions:

The thyroxine content of a given thyroid preparation does not permit a definite conclusion as to its calorigenic action. The iodine content seems to be a better guide, provided the substance has not been treated chemically, especially not been subjected to hydrolysis.

There is a species difference in the absorption of thyroxine through the intestinal canal of the human individual and the rat, respectively: within the experimental error, dissolved thyroxine is completely absorbed by the intestine of the rat.

Isodynamic doses of various derivatives and preparations of thyroid have different but characteristic effects on the pulse rate of the thyroidectomized rat, ranging from a very slight increase (30 beats) to an increase of 175 beats per minute above the initial value of 180 to 200 (122) (123).

From his results it appears that the response at higher levels of metabolism is minimal and therefore unfavorable to fine discrimination of effectiveness.

These various results indicate that natural combinations of thyroxine in peptide chains are more potent than their inherent thyroxine would be alone. Salter and Pearson (140) demonstrated this fact by breaking thyroglobulin down with pepsin and then resynthesizing a part with pepsin to form an artificial protein or "plastein." The activity of the peptone (containing iodine chiefly in diiodotyrosine form) was increased sevenfold by the reverse synthesis (137). Kendall (86) has suggested that the presence of the peptide chain prevents thyroxine from escaping (to the extent of 75 per cent) from the body, and thus increases its effectiveness. This explanation would relieve one of the embarrassments of explaining activity in diiodotyrosine. Clutton, Harington and Yuill have prepared a synthetic combination of protein with thyroxine (21), but its activity has not yet been assayed.

IV. Physiological iodine excretion. *Urinary Iodine.* Urinary iodine excretion depends upon the iodine intake in food, water and air. Thus, in a district with 0.09 gamma per cent in drinking water, the 24-hour excretion was 47 gamma, whereas in another district with 8.9 gamma per cent in the water it was 185 gamma (133). Consequently, it is more profitable to study excretions in districts where water and flora contain only moderate concentrations of iodine. Under

stable conditions in such districts, individual variation is small—not over 10 to 15 gamma in 24 hours. Normally, about half of the total iodine leaves the body by way of the urine (24). A few representative observations follow.

In Columbus, Ohio, 9-year-old children excrete approximately 41 gamma daily and adults 50 gamma (31). In Lwów, values below 20 gamma are found only in goitrous individuals, and values above 50 gamma frequently accompany thyrotoxicosis (36, p. 114). Nevertheless, 70 gamma occasionally may be found in the urine of normal individuals. In Germany, Isenbruch (82) found 6 gamma per cent iodine concentration in the urine of nursing infants. Goats (27) excrete 21 gamma daily and dogs (151) (153) between 8 and 67 gamma, depending on the animal's size. In cattle enjoying an iodine-rich fodder, however, urinary excretion may reach 600 gamma (141). According to McClendon (116), twice as much iodine may be excreted by day as by night. Indeed, 24-hour urine samples are essential in careful studies of iodine metabolism because of hourly variations (143). No doubt, diurnal assimilation of exogenous iodine is largely responsible for such fluctuations. In fasting animals excretion is more uniform, and declines but slowly.

Most, if not all, of the urinary iodine is present as iodide. No thyroxine can be detected in the urine (50), but whether any diiodotyrosine is present normally is not yet clear.

Fecal Iodine. Because almost no iodine is excreted in the feces of fasting men, it seems clear that fecal iodine is almost entirely of exogenous origin. When large doses of thyroxine are administered intravenously the large bowel actively excretes part of the iodine (147). The bile also contributes to fecal iodine, because the iodine in it may increase from 9.2 gamma per cent, fasting, to 45 gamma per cent after feeding (42) (40). A large part of the biliary iodine is reabsorbed so that there is a "circulation" of iodine analogous to the "circulation" of the bile salts. Of course, unabsorbed iodine in foodstuffs also contributes to the fecal iodine concentration. Consequently, in diarrhea more iodine is eliminated, along with unabsorbed foodstuffs, whereas in constipation relatively little iodine is eliminated. On a routine diet, Scheffer (143) (144) found only from 2 to 10 gamma iodine per 24 hours, namely, 3 to 9 per cent of the total iodine excretion. Cole and Curtis (24) found variable but larger amounts, i.e., from 6 to 27 per cent of the intake.

In animals, fecal iodine appears to be small, as judged from the following values for 24-hour excretion: for the hog, 1.8 gamma (141);

for the goat, 3 gamma (27); for the rabbit, 9 to 12 gamma (36) (171). On the contrary, in cows eating large amounts of iodine-containing fodder, Fellenberg (56) found as much as 1300 gamma per 24 hours.

The iodine excretion of normal individuals is increased by factors, like exercise, which augment total metabolism. After exertion, urinary excretion may reach double the control value (116), provided excessive sweating does not divert iodine elimination to the skin, as often happens. It is possible that the increased blood and urinary iodine is due to thyroid secretion (36) (171). Kommerell (92) found no increase in basal metabolism after exercise in thyroidectomized animals. This phenomenon deserves further study, in view of the prevailing opinion that the thyroid exerts its effect only after a latent period.

Other factors which increase total metabolism also increase iodine elimination. For example, it is said that mental excitement alone may double the excretion (56) (167). Likewise, a fever of 39°C. may treble the excretion (96). Very high iodine excretions—as much as 3000 gamma daily—have been observed after severe operations like thoracoplasties (30) (29), and there is an associated hyperiodemia which begins within a few minutes after operation and lasts for several days. McCullagh (117) believes that this extra iodine comes from the thyroid, whereas Curtis and Phillips believe it to be of extrathyroidal origin. The high iodine elimination of thyroid disease will be discussed in another publication (171).

Excretion by Other Routes. The iodine in milk, in sweat and in saliva has been discussed in Section II. Under normal living conditions near the seacoast, these media play a minor part in the total iodine excretion.

Courrier and Aron (26) believe that the thyroid hormone can be transmitted through the milk of bitches and so affect the histological status of the thyroids of puppies. The possible amount of thyroxine so transmitted has been estimated by chemical analysis and, if present at all, must be exceedingly small (44).

Fasting Iodine Excretion. Fasting has relatively little effect on the total excretion of iodine in a well nourished individual. To be sure, the fecal iodine rapidly disappears; only 0.4 gamma per day is found. The iodine reserves, however, maintain a normal urinary excretion for many days or weeks, depending on the original richness of the diet in iodine. The average "endogenous" urinary iodine excretion was found by Sturm (151) to be 18 gamma in dogs which had fasted for four days. In thyroidectomized dogs, however, fasting produces a rapid, marked

drop, and the total excretion may approach zero. Presumably the greater part of endogenous iodine elimination is linked up with thyroid activity, directly or indirectly. Of course, after a depletion of iodine reserves by fasting, restoration of a normal diet is accompanied by a temporary retention which causes a negative iodine balance.

REFERENCES

(1) Abelin, I. Arch. f. exper. Path. u. Pharmakol. **171**: 443, 1933.
(2) Abelin, I. Arch. f. exper. Path. u. Pharmakol. **181**: 250, 1936.
(3) Asimoff, G., E. Estrin and S. Miletzkaja. Ztschr. f. d. ges. exper. Med. **76**: 409, 1931.
(4) Barger, G. Ergebn. d. Physiol. **27**: 780, 1928.
(5) Barnes, B. O. and J. G. Bueno. Am. J. Physiol. **103**: 570, 1933.
(6) Barnes, B. O., A. J. Carlson and A. M. Riskin. Am. J. Physiol. **98**: 86, 1931.
(7) Bauer, H. and E. Strauss. Biochem. Ztschr. **284**: 197, 1936.
(8) Baumann, E. Ztschr. f. physiol. Chem. **22**: 1, 1896–97.
(9) Baumann, E. München. med. Wchnschr. **43**: 398, 476, 1896.
(10) Bier, A. and W. Roman. Ztschr. f. klin. Med. **118**: 15, 1931.
(11) Blum, F. and E. Strauss. Ztschr. f. physiol. Chem. **127**: 199, 1923.
(12) Böe, J. and A. W. Elmer. Biochem. Ztschr. **240**: 187, 1931.
(13) Boothby, W. M. and E. J. Baldes. J. Pharmacol. and Exper. Therap. **25**: 139, 1925. Also, Proc. Staff Meet., Mayo Clin. **1**: 166, 1926.
(14) Brand, E. with B. Kassell. J. Biol. Chem., Soc. Proc. **128**: xi, 1939.
(15) Bubnow, N. A. Ztschr. f. physiol. Chem. **8**: 1, 1884.
(16) Bürger, M. and W. Möbius. Klin. Wchnschr. **13**: 1349, 1934.
(17) Canzanelli, A., C. R. Harington and S. S. Randall. Biochem. J. **28**: 68, 1934.
(18) Cavett, J. W. J. Biol. Chem. **114**: 65, 1936.
(19) Chatin, A. Compt. rend. Acad. d. sc. **36**: 652, 1853.
(20) Chidester, F. E. Arch. internat. de pharmacodyn. et de thérap. **48**: 354, 1934.
(21) Clutton, R. F., C. R. Harington and M. E. Yuill. Biochem. J. **32**: 1119, 1938.
(22) Cohn, B. N. E. Arch. Int. Med. **49**: 950, 1932.
(23) Cohn, E. J., W. T. Salter and R. J. Ferry. J. Biol. Chem., Soc. Proc. xxxii, **123**: 24, 1938.
(24) Cole, V. V. and G. M. Curtis. J. Nutrition **10**: 493, 1935.
(25) Condorelli, L. Riv. di pat. sper. **8**: 197, 1937.
(26) Courrier, R. and M. Aron. Compt. rend. Soc. de biol. **100**: 839, 1929.
(27) Courth, H. Biochem. Ztschr. **238**: 162, 1931.
(28) Courtois, M. B. Ann. de chim. **88**: 310, 1813.
(29) Curtis, G. M. and L. E. Barron. Surgery **1**: 92, 1937.
(30) Curtis, G. M. and F. J. Phillips. J. Clin. Investigation **13**: 777, 1934.
(31) Curtis, G. M. and I. D. Puppel. Arch. Int. Med. **60**: 498, 1937.
(32) Cushny, A. R. A text-book of pharmacology and therapeutics. Philadelphia, Lea & Febiger, 1928.

(33) Davis, C. B., G. M. Curtis and V. V. Cole. J. Lab. and Clin. Med. **19**: 818, 1934.
(34) Dodds, E. C., W. Lawson and J. D. Robertson. Lancet, **2**: 608, 1932.
(35) Dorff, G. B. Am. J. Dis. Child. **48**: 1316, 1934.
(36) Elmer, A. W. Iodine metabolism and thyroid function. London, Oxford Univ. Press, 1938.
(37) Elmer, A. W. Paris méd. **26**: 433, 1936.
(38) Elmer, A. W. Deutsch. Arch. f. klin. Med. **174**: 390, 1933.
(39) Elmer, A. W. Deutsch. Arch. f. klin. Med. **174**: 449, 1933.
(40) Elmer, A. W. and Z. Luczynski. Compt. rend. Soc. de biol. **115**: 647, 1933.
(41) Elmer, A. W. and Z. Luczynski. Compt. rend. Soc. de biol. **115**: 1717, 1934.
(42) Elmer, A. W. and Z. Luczynski. Compt. rend. Soc. de biol. **114**: 1340, 1933.
(43) Elmer, A. W., Z. Luczynski and M. Scheps. Compt. rend. Soc. de biol. **115**: 1714, 1934.
(44) Elmer, A. W. and W. Rychlik. Compt. rend. Soc. de biol. **115**: 1719, 1934.
(45) Elmer, A. W. and W. Rychlik. Compt. rend. Soc. de biol. **117**: 530, 1934.
(46) Elmer, A. W., W. Rychlik and M. Scheps. Compt. rend. Soc. de biol. **117**: 533, 1934.
(47) Elmer, A. W. and M. Scheps. Compt. rend. Soc. de biol. **118**: 1370, 1935.
(48) Elmer, A. W. and M. Scheps. Compt. rend. Soc. de biol. **118**: 1374, 1935.
(49) Elmer, A. W. and M. Scheps. Compt. rend. Soc. de biol. **114**: 350, 1933; Compt. rend. Soc. de biol. **115**, 968, 1934.
(50) Elmer, A. W. and M. Scheps. Acta med. Scadinav. **82**: 126, 1934.
(51) Eppinger, E. and W. T. Salter. Am. J. Med. Sc. **190**: 649, 1935.
(52) Eufinger, H. and W. Schulte. Arch. f. Gynäk. **152**: 479, 1933.
(53) Fashena, G. J. and V. Trevorrow. J. Biol. Chem. **114**: 351, 1936.
(54) v. Fellenberg, T. Biochem. Ztschr. **174**: 355, 1926.
(55) v. Fellenberg, T. Ergebn. d. Physiol. **25**: 176, 1926.
(56) v. Fellenberg, T. Biochem. Ztschr. **184**: 85, 1927.
(57) Fitz, R. and R. S. Hunt. Tr. Assn. Am. Phys. **52**: 24, 1937.
(58) Foot, N. C., L. E. Baker and A. Carrel. J. Exper. Med. **70**: 39, 1939.
(59) Foster, G. L., W. W. Palmer and J. P. Leland. J. Biol. Chem. **115**: 467, 1936.
(60) Freud, J. and E. Laquer. Acta brev. Neerland. **6**: Nr. 7, 1936.
(61) Gaddum, J. H. J. Physiol. **68**: 383, 1929–30.
(62) Glimm, E. and J. Isenbruch. Biochem. Ztschr. **207**: 368, 1929.
(63) Grab, W. Klin. Wchnschr. **12**: 1102; 1637, 1933.
(64) Gudernatsch, J. F. Arch. f. Entwcklngsmechn. d. Organ. **35**: 457, 1912.
(65) Gutman, A. B., E. N. Benedict, B. Baxter and W. W. Palmer. J. Biol. Chem. **97**: 303, 1932.
(66) Gutzeit, K. and G. W. Parade. Ztschr. f. klin. Med. **133**: 503, 1937–1938.
(67) Gutzeit, K. and G. W. Parade. Ztschr. f. klin. Med. **135**: 158, 1938.
(68) Hahn, A. and A. Schürmeyer. Klin. Wchnschr. **11**: 421, 1932.
(69) Harington, C. R. The thyroid gland, its chemistry and physiology. London, Oxford Univ. Press, 1933.

(70) HARINGTON, C. R. Lancet, **1**: 1199, 1261, 1935.
(71) HARINGTON, C. R. AND G. BARGER. Biochem. J. **21**: 169, 1927.
(72) HARINGTON, C. R. AND S. S. RANDALL. Biochem. J. **23**: 373, 1929.
(73) HARINGTON, C. R. AND W. T. SALTER. Biochem. J. **24**: 456, 1930.
(74) HEIDELBERGER, M. AND W. W. PALMER. J. Biol. Chem. **101**: 433, 1933.
(75) HEIDELBERGER, M. AND T. SVEDBERG. Science **80**: 414, 1934.
(76) HEKTOEN, L., P. H. KANAI AND L. R. DRAGSTEDT. J. A. M. A. **84**: 114, 1925.
(77) HENZE, M. Ztschr. f. physiol. Chem. **38**: 60, 1903; ibid. **51**: 64, 1907.
(78) HERTZ, S., A. ROBERTS, J. H. MEANS AND R. D. EVANS. Am. J. Physiol. **128**: 565, 1940.
(79) HERXHEIMER, H., E. MISLOWITZER AND L. STANOYÉWITCH. Compt. rend. Soc. de biol. **115**: 886, 1934.
(80) HIRSCH, O. Deutsch. Arch. f. klin. Med. **168**: 331, 1930.
(81) HUTCHISON, R. J. Physiol. **20**: 474, 1896.
(82) ISENBRUCH, J. H. Über den Jodgeholt menschlicher Organe. Diss. Danzig, 1929.
(83) JUSTUS, J. Virchow's Arch. **170**: 501, 1902; Ibid. **176**: 1, 1904.
(84) KENDALL, E. C. J. A. M. A. **64**: 204, 1915.
(85) KENDALL, E. C. Thyroxine. Chemical Catalogue Co., New York, 1929.
(86) KENDALL, E. C. J. A. M. A. **104**: 978, 1935.
(87) KENDALL, E. C. AND F. S. RICHARDSON. J. Biol. Chem. **43**: 161, 1920.
(88) KENDALL, E. C. AND D. G. SIMONSEN. J. Biol. Chem. **80**: 357, 1928.
(89) KLEIN, W., A. PFEIFFER AND G. HERMANN. Biochem. Ztschr. **225**: 344, 1930.
(90) KÖGL, F. AND H. ERXLEBEN. Ztschr. f. physiol. Chem. **258**: 57, 1939.
(91) KOMMERELL, B. Arch. f. exper. Path. u. Pharmakol. **161**: 141, 1931.
(92) KOMMERELL, B. Pflüger's Arch. **227**: 1, 1931.
(93) KRAYER, O. Arch. f. exper. Path. u. Pharmakol. **128**: 116, 1928.
(94) LEIPERT, T. Mikrochim. Acta. **3**: 73, 1938.
(95) LEIPERT, T. Biochem. Ztschr. **270**: 448, 1934.
(96) LEIPERT, T. Biochem. Ztschr. **293**: 99, 1937.
(97) LELAND, J. P. AND G. L. FOSTER. J. Biol. Chem. **95**: 165, 1932.
(98) LERMAN, J. Personal communication, 1939.
(99) LERMAN, J. AND J. H. MEANS. Am. J. Med. Sc. **181**: 745, 1931.
(100) LERMAN, J. AND W. T. SALTER. J. Clin. Investigation **16**: 678, 1937.
(101) LERMAN, J. AND W. T. SALTER. J. Clin. Investigation (Proc.) **18**: 493, 1939.
(102) LERMAN, J. AND W. T. SALTER. Endocrinology **18**: 317, 1934.
(103) LERMAN, J. AND W. T. SALTER. Endocrinology, **25**: 712, 1939.
(104) LIPSCHITZ, W. Klin. Wchnschr. **8**: 116, 1929.
(105) LOEB, L. Ann. Int. Med. **9**: 13, 1935.
(106) LOEB, O. Arch. f. exper. Path. u. Pharmakol. **56**: 320, 1907.
(107) LÖHR, H. Arch. f. exper. Path. u. Pharmakol. **180**: 344, 1936.
(108) LUDWIG, W. AND P. v. MUTZENBECHER. Ztschr. f. physiol. Chem. **244**: iv, 1936.
(109) LUNDE, G., K. CLOSS AND K. WÜLFERT. Biochem. Ztschr. **206**: 248, 1929.
(110) LUNDE, G., K. CLOSS AND O. C. PEDERSEN. Biochem. Ztschr. **206**: 261, 1929.

(111) MARINE, D. J. Biol. Chem. **22:** 547, 1915.
(112) MARINE, D. AND C. H. LENHART. Arch. Int. Med. **3:** 66, 1909.
(113) MARTIN, N. H. Pharm. J. **89:** 144, 169, 1912.
(114) MAURER, E. AND H. DUCRUE. Biochem. Ztschr. **193:** 356, 1928.
(115) MCCLENDON, J. F. Iodine and the incidence of goiter. Minneapolis, Univ. of Minnesota Press, 1939.
(116) MCCLENDON, J. F., R. E. REMINGTON, H. v. KOLNITZ AND R. RUFE. J. Am. Chem. Soc. **52:** 541, 1930.
(117) MCCULLAGH, D. R. Cleveland Clin. Quart. **2:** 15, 1935.
(118) MCCULLAGH, E. P. AND D. R. MCCULLAGH. Arch. Int. Med. **57:** 1061, 1936.
(119) MEANS, J. H. The thyroid and its diseases. J. B. Lippincott Co., Philadelphia, 1937.
(120) MEANS, J. H. AND J. LERMAN. Ann. Int. Med. **12:** 811, 1938.
(121) MEANS, J. H., J. LERMAN AND W. T. SALTER. J. Clin. Investigation **12:** 683, 1933.
(122) MEYER, A. E. Personal communication.
(123) MEYER, A. E. AND M. YOST. Endocrinology **24:** 806, 1939.
(124) MOEBIUS, W. AND F. A. NOLTE. Ztschr. f. klin. Med. **128:** 174, 1935.
(125) MÜLLER, C. AND T. v. FELLENBERG. Mitt. a. d. Grenzgeb. d. Med. u. Chir. **42:** 661, 1932.
(126) v. MUTZENBECHER, P. AND W. LUDWIG. Ztschr. f. physiol. Chem. **258:** 195, 1939.
(127) PERKIN, H. J. AND F. H. LAHEY. Arch. Int. Med. **65:** 882, 1940.
(128) PERKIN, H. J. AND B. R. BROWN. Personal communication.
(129) PERKIN, H. J., F H. LAHEY AND R. B. CATTELL. New England J. Med. **214:** 45, 1936.
(130) PERKIN, H. J. AND F. H. LAHEY. New England J. Med. **216:** 501, 1937.
(131) PINCUSSEN, L. AND W. ROMAN. Biochem. Ztschr. **216:** 336, 1929.
(132) RABOURDIN, M. Compt. rend. Acad. d. sc. **31:** 784, 1850.
(133) REITH, J. F. Schweiz. med. Wchnschr. **63:** 791, 1933.
(134) RIENHOFF, W. F., JR. Medicine **10:** 257, 1931.
(135) SAEGESSER, M. Schilddrüse Jod und Kropf, p. 94, Basel, 1939.
(136) SALTER, W. T. AND J. LERMAN. Tr. Assn. Am. Phys. **53:** 202, 1938.
(137) SALTER, W. T. AND J. LERMAN. Endocrinology **20:** 801, 1936.
(138) SALTER, W. T., J. LERMAN AND J. H. MEANS. J. Clin. Investigation **12:** 327, 1933.
(139) SALTER, W. T., J. LERMAN AND J. H. MEANS. J. Clin. Investigation **14:** 37, 1935.
(140) SALTER, W. T. AND O. H. PEARSON. J. Biol. Chem. **112:** 579, 1936.
(141) SCHARRER, K. Chemie und Biochemie des Jods. F. Enke, Stuttgart, 1928.
(142) SCHARRER, K. AND J. SCHWAIBOLD. Biochem. Ztschr. **195:** 228, 1928; ibid. **180:** 307, 1927.
(143) SCHEFFER, L. Biochem. Ztschr. **259:** 11, 1933.
(144) SCHEFFER, L. Ztschr. Klin. Med. **132:** 343, 1937.
(145) See discussion by G. B. WALLACE AND B. B. BRODIE. J. Pharmacol. and Exper. Therap. **61:** 397, 412, 1937.
(146) SCHITTENHELM, A. AND B. EISLER. Ztschr. f. d. ges. exper. Med. **80:** 569, 1932.

(147) SCHITTENHELM, A. AND B. EISLER. Ztschr. f. d. ges. exper. Med. **80**: 580, 1932.
(148) SCHITTENHELM, A. AND B. EISLER. Ztschr. f. d. ges. exper. Med. **86**: 275, 1933.
(149) SCHNEIDER, E. AND E. WIDMANN. Deutsch. Ztschr. f. Chir. **231**: 305, 1931.
(150) STRAUSS, E. AND R. GRÜTZNER. Ztschr. f. physiol. Chem. **112**: 167, 1920.
(151) STURM, A. Deutsch. Arch. f. klin. Med. **161**: 129, 1928.
(152) STURM, A. Ztschr. f. d. ges. exper. Med. **74**: 514, 1930.
(153) STURM, A. Ztschr. f. d. ges. exper. Med. **93**: 490, 1934.
(154) STURM, A. AND B. BUCHHOLZ. Arch. klin. Med. **161**: 227, 1928.
(155) STURM, A. AND R. SCHNEEBERG. Ztschr. f. d. ges. exper. Med. **86**: 665, 1933.
(156) SVEDBERG, T. Personal communication.
(157) SZÁSZ, E. Med. Klin. Nr. 47, 1933.
(158) THOMPSON, W. O., J. M. ALPER, P. K. THOMPSON AND L. F. N. DICKIE. J. Clin. Investigation **13**: 29, 1934.
(159) THOMPSON, W. O., P. K. THOMPSON, S. G. TAYLOR, S. B. NADLER AND L. F. N. DICKIE. J. A. M. A. **104**: 972, 1935.
(160) TIEDCKE, C. Klin. Wchnschr. **12**: 383, 1933.
(161) TREVORROW, V. J. Biol. Chem. **127**: 737, 1939.
(162) TURNER, R. G. Proc. Soc. Exper. Biol. and Med. **30**: 1401, 1933.
(163) UHLENHUTH, E. Biol. Bull. **42**: 143, 1922.
(164) VEIL, W. H. AND A. STURM. Deutsch. Arch. f. klin. Med. **147**: 166, 1925.
(165) WHEELER, H. J. AND G. S. JAMIESON. Am. Chem. J. **33**: 365, 1905.
(166) WILLIAMSON, G. S., I. H. PEARSE AND H. M. CUNNINGTON. J. Path. and Bact. **31**: 255, 1928.
(167) WITTKORNER, E., W. SCHERINGER AND E. BAY. Klin. Wchnschr. **11**: 1186, 1932.
(168) ZAWADOWSKY, B. M. Endokrinologie **12**: 81, 1933.
(169) ZAWADOWSKY, B. M. AND G. ASIMOFF. Pflüger's Arch. **216**: 65, 1927.
(170) ZAWADOWSKY, B. M. AND Z. M. PERLMUTTER. Arch. f. Entwcklngsmechn. d. Organ. **109**: 210, 1927.
(171) SALTER, W. T. Iodine and Endocrine Balance. Cambridge, Harvard University Press, 1940.

THE RÔLE OF POTASSIUM IN PHYSIOLOGICAL PROCESSES

W. O. FENN

Department of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

We are still far from being in a position to present the known facts concerning the physiology of potassium from the point of view of any well developed theory of potassium behavior. Nor is it possible in these few pages to describe or even mention more than a very few of the infinite number of "effects" of K which have been observed in connection with almost all known physiological processes. The various attempts which have been made to explain these effects of K in terms of the fundamental properties of membranes and cells would fill an entire review. An arbitrary selection must therefore be made of a few aspects of the subject which seem to be of particular interest to vertebrate physiology.

As is well known potassium is of the soil and not the sea; it is of the cell but not the sap. Macallum (253) has discussed the various explanations which have been advanced to explain the relative scarcity of potassium in the sea. The reason for the great abundance of potassium in living cells is quite different and still unknown. It is not within the scope of this review to discuss in detail the various theories of potassium accumulation. It will suffice to state here that body cells in general appear to be more or less freely permeable to potassium which presumably stays where it is because no other cation can get in to take its place and because the anions with which it is combined are unable to escape.

The permeability of tissue cells to potassium is shown by the rapid disappearance from the blood stream of injected potassium. A more direct proof is afforded by injection of the artificial radioactive isotope of potassium. In such experiments Joseph, Cohn and Greenberg (205) have shown that soon after injection the marked K reaches a high concentration in the liver after which it slowly moves out of the liver and the concentration in the muscles and in the whole blood slowly increases. A few observations have been reported which show (144) that the marked K is about equally concentrated in liver and muscle

of rabbits after 24 hours but indicate that only about 8 per cent of the muscle K has exchanged and only about 3 per cent of the red cell K. It has been found, in rats, however, (298) that after 10 hours all the muscle K and half of the red cell K has exchanged with the marked atoms. Of greater importance is the fact that after 1 hour a larger fraction of the total K in the liver is radioactive than in the plasma or the carcass as a whole (298). In explanation of this fact it may be postulated that the visceral organs immediately after injection take up or ingest an isotonic solution of potassium of high radioactivity and later give it up slowly by cation exchange to the plasma and thence to the other tissues like the muscles, the red cells and brain. The muscles, therefore, ingest very little excess K but exchange their K fairly rapidly with plasma K. Red cells and brain neither ingest nor exchange K rapidly. All the visceral organs ingest K rapidly but exchange it with the blood about as rapidly as muscles. In any event even the red cells are more or less permeable to K (in rats). Their permeability to Na has recently been shown by similar experiments with radioactive Na (81). Chemical evidence of permeability of red cells to K is not lacking (214) (160), but is still exceptional (308).

Nevertheless, in spite of this demonstrated permeability to K and Na the Donnan membrane theory assuming complete cation impermeability, appears to explain the electrolyte equilibrium in red cells satisfactorily.

The theory of the electrolyte equilibrium across the cells of muscles and other tissues seems to be in a somewhat similar predicament. The most acceptable theory to date has been based on the assumption that the cells are permeable to K but not to Na and are impermeable to anions (281). A recent modification of this theory, which seems to be a considerable improvement, is that described in the preliminary communication of Conway and Boyle (83) who propose that the membrane is permeable to K and the monovalent anions but impermeable to Na and to all other anions.

The most obvious difficulty with this theory is the finding (164) that the large amounts of necessarily intracellular Na found in muscles of rats raised on a low K diet readily exchanges with injected radioactive Na. Here again, in spite of this demonstrated permeability to both K and Na, the theory of Conway and Boyle seems to explain satisfactorily a good many features of the muscle electrolyte balance. Some of these will be referred to later.

The importance of K in the interior of cells. Since all animal cells contain a large amount of K it is pertinent to inquire whether this serves any special function for which Na would not be equally suitable.

In red blood cells it is evident that K plays no essential part which cannot be served equally well by Na, for the corpuscles of cats and dogs contain little more K than the plasma and still function adequately as oxygen carriers. Clark (77) asserts that the frog heart can continue to beat after losing half of its K providing that a trace of K is present in the perfusion fluid. Muscle cells have been observed in K-deficient rats which still contracted even though half the potassium inside the cells had been replaced by Na (165). Such rats, however, are not healthy and it is impossible to say at present to what extent the contractility of the muscles was altered by this change of cation. Moreover it is possible that the potassium in the myofibrils themselves was not lost but only the potassium in the sarcoplasm. The greater solubility of muscle proteins in potassium as compared to sodium salts (162) suggests another possible advantage for a muscle with K as the chief cation. In the liver cells it has been shown that glycogen cannot be deposited unless accompanied by appropriate amounts of K and water (114) but it is probable that Na would serve equally well if it could penetrate the cell. The chief if not the only definite advantage of potassium depends upon its greater mobility, a difference which exists even in aqueous solutions but which is intensified 4.5 times (272) within a collodion membrane. This may make possible the separation of Na and K by the cell membrane. The resulting differences in the concentration of K cause potential differences across the cell membrane and probably account for the properties of excitability and conductivity. The predominant effect of K as compared to other ions upon potential differences in muscles confirms this interpretation. (For references see previous review, 112.) Since no other cation can entirely replace K in the interior of cells without interfering to some extent with the cell function this particular cation must be granted a certain degree of indispensability for life. Even rubidium with a mobility still higher than that of K cannot serve the same purpose.

Isotopes of K. Interest in potassium has been much enhanced in recent years by the discovery of the natural existence of the rare radioactive K^{40} isotope in addition to the heavier K^{41} and the ordinary K^{39} isotopes. This finding in turn led to a recalculation of the half life of this radioactive isotope as 1.67×10^8 years, a much lower value than was previously obtained when all the radioactivity was attributed to the 579 times more abundant K^{41} isotope (44). The K^{40} isotope must disintegrate into Ca^{40} and from the K^{40}/Ca^{40} ratio in the earth's crust the age of the earth has been estimated as 3×10^9 years, a figure in fair agreement with values obtained by other methods (44). Moreover it

appears that the heat of this disintegration in early times must have played an important rôle in maintaining the temperature of the earth. Since all the Ca on the earth came originally from K^{40} it may be said that the biologically important ratio of these two physiological antagonists has been continuously changing since the origin of life.

The possiblity that K might be of importance in cells because of the special radioactive properties of its K^{40} isotope, as originally suggested by Zwaardemaker (410), has been thoroughly disproved. It can be calculated that in heart muscle there is only about 1 β ray produced per gram of muscle per second from the K^{40} isotope. Failure of the heart to beat in a K-free perfusate can hardly be due to lack of such discharges because the K content of the muscle cells themselves is not thereby diminished, nor is the K content of the heart as a whole appreciably changed. In addition Glazko and Greenberg (138) have shown recently that such a heart cannot be made to beat by replacing K by radioactive Na although the β ray activity is thereby increased much above normal. Hamilton and Alles (146) could find no physiological effects from the injection of artificial radioactive isotopes in spite of the great increase in the number of β ray discharges. Further review of older evidence against this theory is no longer profitable.

The related question as to whether the abundance ratios of the K isotopes are the same in living tissues as in the ocean or the earth's crust is still under investigation. Brewer (43) by means of the mass spectrograph has studied the abundance of K^{41} from different sources and reports a slight excess in bone marrow, spleen and kelp and a slight deficiency in rat tumors and auricle (238). These small differences amount to less than 2 per cent and have not yet been confirmed. Loring and Druce (251, 95) prepared KCl from 200 potato plants and found its molecular weight to be 40.04 ± 0.1 indicating an excess of the heavier isotopes. Further confirmation of this finding is desirable, especially since Brewer (45) found relatively less K^{41} in potato vines than in other sources of K. Ernst (103) concluded that biological K had a greater effect on a photographic plate than inorganic K indicating thus an accumulation of the radioactive isotope K^{40} in living tissues. Pohlmann (310, 311) however extracted and purified K from tissues by precipitation as the perchlorate and determined the radioactivity with a Geiger-Müller counter. He concluded that K^{40} was not more than 5 per cent more abundant than in control K samples. Similar data have been obtained (239) for rabbit muscle K, for tumor K (236) and in the writer's laboratory (unpublished) for K from human ashes.

A. and M. Lasnitzki (237) compared the effects of substituting biological K extracted from maize, casein and agar for mineral K in the diets of mice, but their results were contradictory and probably indicated no differences.

Certainly to date the literature contains no evidence that the isotopic composition of biological K is sufficiently different from the normal to interfere with the successful use of the radioactive isotopes as tracers.

The potassium balance of the body. The whole body of an adult rat contains 0.25 per cent potassium (243) (166) (245). The K content of the human body has been variously estimated as 0.11 per cent (252) and 0.35 per cent (338). Assuming an average value of 0.25 per cent it may be estimated that the body of a 70 kgm. man contains about 175 grams of K. Of this amount the blood will contain about 8 grams, the blood plasma 0.3 gram and the total extracellular space about 3 grams.

Most of the ingested K is excreted in the urine but small amounts appear regularly in the feces (308). In two babies (340) 8 to 9 per cent of the ingested K was excreted in the feces the absolute amount increasing in proportion to the intake. According to figures of Wiley and Wiley (383) the feces contain regularly about 0.3 gram of K daily, or about $\frac{1}{6}$ of the daily intake of their subject, but only negligible amounts of Na.

The average intake and excretion of K per day for an adult man averages about 3.4 grams (338, p. 371). During a 6-day fast in an 8 year old girl the excretion of K was reduced to 1.08 gram of K accompanied by 10.3 grams of N (128) whereas in a man after 31 days (25) the daily excreted K and N amounted to only 0.6 and 7 grams respectively. During a fast therefore the N/K ratio in the urine has a value of about 10 which is likewise about equal to the ratio of N to K found in normal muscles (128). This indicates therefore a continuous unavoidable loss of K during fasting which is due to the gradual destruction of cells in the endogenous metabolism of the body. After fracture of a limb a similar loss of nitrogen accompanied by potassium has been observed (86).

Since potassium balance can only be maintained by continual excretion in the urine it is not surprising that there should be a large rise of serum K in experimental nephrectomy (297) (156) (261), but not in milder cases of partial ureteral obstruction (98). In human nephritis high K is seen only occasionally in the most severe cases (293) (303) (313) (386). In general, Ca falls slightly possibly because of the high

phosphate which accompanies the high K (409) (408). Usually, however, no disturbance of K is seen even in severe uremia (10) (289). The symptoms of uremia are not therefore due in general to the toxicity of an excess of potassium (1) as has been sometimes suggested. By histochemical methods Menten (270) has found increased amounts of K, particularly in the convoluted tubules, resulting from diuresis or injury.

Dietary potassium. Lack of K in the diet is detrimental and inhibits growth (305) (274) (275) (276) (243) (166) (9) (147). According to Redina (315) rats grow best if the Na/K ratio is 5, the loss of weight resulting from the inadequate diet being increased with either more or less K. The diet used in this case was, however, deficient in vitamins. On an otherwise adequate diet growth can be completely prevented by lack of K and a relatively enormous excess of K can be tolerated without obvious ill effects. Thus Miller (274) has found no harmful effects in rats from a diet containing 14 times as much K as Na.

In addition to showing inhibition of growth by a lack of K, Heppel and Schmidt (166) have studied the effect of such a deficient diet on pregnancy. While litters were born under such conditions they were promptly eaten by the potassium-depleted mother. Some growth (50–60 per cent of normal) was obtained by substituting Rb for K but such rats became so hyperexcitable that they went into convulsions at the sound of a blast of air and they died prematurely. Except for the K deficiency the diet was adequate for normal growth. Rats raised on a low K diet lose as much as half the K of their muscles, replacing it by Na, but the liver remains unchanged in composition (163).

Likewise man loses K on a salt-poor basal diet supplemented with NaCl (385) and the muscle K of rats on a low K diet is made still lower if NaCl is added to the ration, thus providing in excess a cation which can exchange with K (101). The electrolyte composition of the blood does not change, however, on such low salt diets (345). Numerous investigations have established the general principle that by such a cation exchange intake of K can cause a loss of Na and vice versa (34) (242) (275) (136) (384) (32) (213) (210). Likewise a vegetarian diet which is relatively rich in K requires and is normally accompanied by a higher intake of NaCl (366). The same effect can be nicely demonstrated in the isolated dog kidney (199).

The potassium turnover in the blood. In pancreatic juice the concentration of K is about equal to that in blood plasma, i.e., 4 to 5 m. eq. per liter (203) and after the injection of KCl the increase of K concen-

tration in the pancreatic secretion is about equal to the increase in the plasma (21). In other secretions of the gastrointestinal tract, however, the K concentration is greater than that in the plasma in varying degrees, the values (in m.eq. per liter) being 6.2 to 7.2 in secretions of the jejunum, ileum and colon (24), 6.6 in the hepatic bile (317), 14.0 in the secretion of the esophagus (363) and in the gastric juice (12) and 18.9 in the saliva (weighted average—368). Most of the K secreted in this way into the gastrointestinal tract is reabsorbed lower down so that there is a continual circulation of K and other salts through this route. Using the values given above and the volumes of secretions as estimated by Adolph (3) it may be calculated that about 1.25 gram of K circulates through the glands into the gastrointestinal tract and back again in 24 hours, or all the K in the plasma circulates once every 6 hours. Since, moreover, all the K in the plasma is excreted by the kidney every 2 or $2\frac{1}{2}$ hours in addition to some loss in the sweat, it is evident that the turnover is exceedingly rapid and the constancy of the K level in the plasma becomes all the more remarkable.

Potassium by mouth. Ingested K under certain conditions is absorbed more rapidly than Na (198) presumably because the diffusion gradient into the blood is more favorable. The difference between K and Na in this respect cannot be large because no appreciable difference was found in previous investigations (178) (373) (292). After ingestion K is excreted more rapidly than Na (2) indicating that the K capacity of the body is relatively less "elastic" than the Na capacity. For the same reason potassium salts have proved useful as diuretics (213). Ingested K is also taken up rapidly by the tissues, the increase in concentration of K in the plasma being such as to indicate that the K diffuses into all the body water (39). The ingestion of 12 grams of KCl caused a 61 per cent increase in the plasma 2 hours later (299). Such an increase is not toxic. Similar figures were obtained by Wilkins and Kramer (386). According to Osborne (304), curiously enough, there is no comparable increase of plasma K after the ingestion of 20 grams of KI. With K acetate as little as 2.5 grams per day caused a measurable increase of plasma K (329).

Injected potassium and its toxicity. The efficiency of the body in disposing of excess K is best seen when K is injected into the blood. Such potassium rapidly disappears from the blood (46) the residual increment of K in the plasma being such as to indicate again that the injected K might have diffused into all the body water (392) (381) (115). Wilde (381) has found that some of this K comes back into the

blood again temporarily after 30 to 40 minutes. There is evidence that the liver absorbs more than its proportional share of such injected K (115), but most of the injected K is absorbed by muscles since the rate of disappearance from the blood is said not to be influenced by the removal of the kidney, liver and the alimentary canal (189) (190).

Injected potassium is difficult to trace by tissue analysis. This is due *1*, to the considerable error in the analysis for K, and *2*, to the small amount of K which can be injected. In man the maximum amount considered safe to inject intravenously at one time is only 3 to 4 mgm. per kgm. of body weight (337) (237). This is less than 0.2 per cent of the total 2500 mgm. K present in 1 kgm. of body weight. Perhaps the smallest rapidly injected intravenous dose which has been reported as lethal is 8 mgm. of K per kgm. in guinea pigs (11). For a 2 minute injection 50 mgm. of K per kgm. is a more usual figure (8) (379). The slower the rate of injection the larger the amount of K which can be tolerated. With a very slow infusion of isotonic KCl over a period of an hour the K content of the body can be increased by 220 mgm. per kgm. or about 9 per cent of the normal content (34) (299) before the lethal level of 15 m. eq. per liter of serum is reached (391).

A third reason why injected K is difficult to trace in the tissues is *3*, that it is probably taken into the cells as an isotonic K solution, thus causing little or no change in the concentration of K per kgm. of wet weight. There should be a change on a dry weight basis but changes of dry weight by deposition of glycogen or other means are also possible. Liver analyses before and after injection of KCl have suggested that most of the change in composition was due to an intake by 1 kgm. of liver of 23 cc. of cell water containing isotonic K and a loss of 7 cc. of extracellular water (115).

It is noteworthy that this result is at least qualitatively explained by the Conway and Boyle theory which also predicts that injected K should distribute itself more or less equally in all the body water rather than in proportion to the pre-existent concentration of K as required by the Mond and Netter theory. The liver appears to obey the Conway-Boyle theory better than the muscles in this respect.

If K is injected into the arteries it causes a marked vasoconstriction but is less toxic than when injected intravenously where it reaches the heart in larger concentration and may cause a slowing, auriculo-ventricular block, fibrillation and finally cessation of the beat (263) (145) (167). In passing through the capillaries arterially-injected K becomes mixed with a larger amount of blood before reaching the heart and some

of it is absorbed in the tissues, for venous blood under such circumstances has been found to contain less K than arterial blood.

The toxicity of injected K is diminished by simultaneous injection of Ca (391) or of hypertonic NaCl or glucose (8).

Potassium in growth. There is prevalent an opinion that a high growth rate of tissues is related to a high K content. In mice large rapidly growing tumors had a high K and low Ca content while the reverse was true in slowly growing tumors (80). In general K falls and Ca rises with increasing age of the tumor. In the chicken sarcoma (284) there is in general a fall of K and Mg and a rise of Na, Ca and Cl in passing from the normal through the actively growing periphery of the tumor to the necrotic interior and the same changes occur progressively as the tumor ages. In human tumors the K content has been found uniformly higher than in the corresponding normal tissues, and a low K diet has been recommended for cancerous patients (102). The bone marrow has also been reported high in K during the growth of carcinomas in the body (184). Meyer-Dorkin (271) has found some evidence of local stimulation of growth from daily injections of K in mice, and Kaufman and Laskowski (209) have sought to establish some relation between the decrease in the K content of the eye and the decrease in its growth rate, the eye being an organ the function of which is not appreciably changed by age. During pregnancy the K content of the uterus increases with the growth of that organ (390). This is probably due, however, merely to a decrease in the relative amount of non-muscular tissue. In plants also most K is found when growth is rapid (404). A high K content, however, is presumably the result rather than the cause of the rapid growth and there seems to be no evidence that K has any specific effect upon growth except that it supplies an essential component of the new protoplasm.

Rôle of K in muscle contraction. Many investigations have shown that muscular activity leads to a loss of K in exchange for Na. Pertinent literature has been reviewed previously in this journal (112). More recently the main facts have been confirmed in cats (361), in rats (165) and in frogs (58). The expected increase of K in the blood has also been found in isolated perfused muscles (5) (396), in rats (212), in man after exercise (216) and in cats and frogs (author—unpublished). Heppel (165) has shown that the loss of K in muscular activity persists in rats raised on a K-deficient diet and that the percentage loss is much larger in young than in adult rats.

The significance of the loss of K which apparently accompanies all

activity of skeletal muscle is still somewhat problematical and the information concerning it is still incomplete. The loss of K is in general proportional to the duration and the intensity of the contraction although an incomplete tetanus liberates more K than a complete tetanus, —as if rhythmical activity assists in some way in the liberation (114 a). In a series of twitches the rate of loss of K diminishes with time, but so does the amount of tension developed. Possibly the progressive loss of K is one of the factors which causes the intensity of contraction to decrease. The data do not preclude the possibility that after an hour of stimulation some contractions may continue without further net loss of K. Presumably a steady state is reached in which the loss due to contraction is just equal to the gain due to recovery. Such is probably the condition in the heart. The gain of water on stimulation reaches a maximum after 5 to 10 minutes after which time contraction continues without further gain (actually with a loss) of water (165, cf. also 361). So far as we know, however, the activity of skeletal muscle is always accompanied by a loss of K.

These results make it probable that the loss of K is intimately connected in some way with the contractile process or the immediate recovery processes rather than with the neuromuscular transmission of excitation. The magnitude of the loss, amounting in young rats (165) to 30 per cent of the total muscle K, seems much too large to be accounted for in nerve endings only. Nevertheless Reginster (316) reports experiments showing that in frog muscle stimulation though not sufficient to cause a demonstrable actual loss of K from the muscle, may nevertheless cause a definite increase in the amount of K which can be removed from the frozen and pulverized muscle by a single extraction with water, i.e., an increase in free and a decrease in bound potassium. He further showed that this apparent increase in free K has no relation to the contractile process because it persists even after the complete inhibition of contraction by curare. This surprising result needs confirmation but in any event it does not contradict the conclusion that the actual loss of K from active muscle is dependent upon the contraction of the muscle, for when the contraction is inhibited by curare the loss of K also disappears (Wood, Collins and Moe (396) in perfused dog muscle; Fenn, Koenemann and Sheridan (118) in perfused frog muscle).

A loss of K during stimulation has been explained as the result of an increase in permeability, but this theory has encountered various difficulties (112). According to the Conway-Boyle theory such a loss would occur as a result of an increase in the number of permeable anions.

Loss of K during activity of other excitable tissues. In non-medullated nerve a loss of K on stimulation has been found (85) (401). In medullated cat nerve in vitro it has not been possible to demonstrate such a loss (113). In the sympathetic ganglia, however, (365) there is evidence of diminished K content on the stimulated side. It seems not unlikely therefore that excitation permits a surge of potassium ions through the membrane which then escape into the blood unless diffusion is too much delayed by a medullary sheath.

In *Nitella,* Hill and Osterhout (174) have postulated a similar surge of potassium ions through the membrane in explanation of the action potential. They have also found evidence for the existence in the surface of these large plant cells of some substance, possibly a compound of potassium, the presence of which is essential for the irritability of the cell (173) (175). This compound is removed by treatment with distilled water but can be restored by various means including the treatment of the cell with blood serum (307). Direct experimental evidence for a loss of K on stimulation of these cells has not yet been reported.

In the salivary gland Wills and Fenn (388) found no apparent decrease in K content of glands stimulated through the chorda tympani nerve although a decrease was quite evident after pilocarpine stimulation. In this case the loss of K in the saliva cannot be replenished quickly enough from the blood whereas in electrical stimulation the output equals the intake. The behavior of the glands, therefore, is not contrary to the rule that a loss of K is inevitably associated with activity.

Heart muscle like skeletal muscle probably loses K during activity for low K contents have been found in the over-worked hearts of cardiac patients after death (61) (387) (151).

The possiblity of loss of K from smooth muscle or the central nervous system during activity has not been adequately investigated. Pichler (309) however reported no decrease in K content of the frog brain and cord after strychnine as compared to normal or urethanized frogs. After urethane, however, a larger fraction of the total K could be extracted in 96 per cent alcohol.

The rôle of K in neuro-muscular transmission. An increase of both K (loc. cit.) and acetylcholine (87) has been detected in the venous blood from stimulated muscle. The same is true in the heart after vagal stimulation [for K (194) (247) (38) (131); for acetylcholine (250) (121)] and in the superior cervical ganglion [(365) for K; for acetylcholine (55)]. This finding, however, is probably not peculiar to nerve endings for acetylcholine has also been detected coming from stimulated nerve trunks or their cut ends (26) (29) (27) (248), or present in the nerves

in increased amount as a result of stimulation (288). The increasing evidence of this sort makes it likely that this is a normal physiological process in spite of the contrary view which has been expressed (126). Evidence, such as it is, for a loss of K from stimulated nerve trunks has already been cited. These facts do not suggest therefore that the process of conduction across a synapse or myoneural junction is any more hormonal in nature than the process of nerve conduction itself. Nevertheless the concentrations of both K and acetylcholine appear to have important pharmacological effects in the process of intercellular nervous transmission, and it may well be that under different conditions any one of the 3 factors, K, acetylcholine or electrical change, might become by itself the decisive factor in permitting or preventing the passage of excitation. Space does not permit discussion of this question here except as it relates to K.

Potassium possesses certain properties resembling those of acetylcholine which fit it to act as a neurohumor. Brown and MacIntosh (56) have shown that application of K to nerve fibers can cause excitation and Cicardo (73) has produced a contraction of the gastrocnemius muscle by injection of KCl into the sciatic nerve. Buchthal and Lindhard (57) in the isolated muscle fiber of the lizard have found that the application of KCl to the end plate causes a rapid tetanus-like contraction while on the muscle fiber itself there is no effect except for the contracture produced by higher concentrations of K. Brown (54) has observed the twitch-like contraction of cat muscle caused by close arterial injections of KCl followed by an electrically "silent" contracture. Moreover, Szent-György, Bacq and Goffart (354) have reported an experiment in which the hind legs of two frogs were perfused in series; contraction of the muscles of the first frog was followed after a suitable interval by irregular contractions of the muscles of the second frog. The result was obtained only when veratrine was present which sensitizes muscle to K (17) and the experiment succeeded in the absence of eserine so that any acetylcholine would have been destroyed. The authors conclude that K is the transmitting agent in this case.

The writer has sometimes found the concentration of K in the blood from stimulated frog legs higher than that in the arterial blood by 100 per cent and in the presence of veratrine this increase in concentration might well be sufficient to cause stimulation. In the experiment of Szent-György et al. (354), therefore, K probably did act as the agent transmitting excitation, but this is no proof that K has this function normally. The fact that curare abolishes nerve muscle transmission

but does not abolish the stimulating effect of K (73) (54) seems to rule out this possiblity. Brown and Feldberg (55) have advanced the same argument against the contention of Eccles (97) that K is the transmitting agent in the superior cervical ganglion.

However, even though K cannot properly be regarded as a humoral agent for neuromuscular transmission it undoubtedly plays an important rôle in the neuromuscular junction or synapse. Thus the injection of KCl (389) (110) may serve to reëstablish contractions from nerve stimulation in a muscle previously paralyzed by curare. The striking effects of K in curing or preventing an attack of familial periodic paralysis (280) (171) (7) are to be interpreted in this sense. During spontaneous attacks of this disease the K in the blood regularly decreases (129) (4) (130). The attacks may be induced by procedures tending to lower blood K such as injection of adrenalin, insulin, or of sugar (339) (400) or a high carbohydrate meal. Injection of $CaCl_2$ aggravates the attacks, whereas injection of K or muscular exercise (which liberates K) tends to prevent them. (Patients testify that when they feel an attack coming on they can sometimes "walk it off" (280).)

Some favorable effects of K have also been observed in myasthenia gravis (372) where the dramatic effect of prostigmin indicates clearly that the difficulty is myoneural in its location. This effect may be due to a liberation of acetylcholine by the K or to the ability of K to inhibit cholinesterase (269). However Minot (278) has found guanidine also effective in relieving myasthenia gravis although it has no inhibitory effect upon cholinesterase. Both guanidine and potassium (and acetylcholine) contractions (132) of muscle are inhibited by Ca, and guanidine and potassium are synergistic in their effect on muscle (125) (399) (320). It is therefore possible that guanidine resembles K in its ability to liberate acetylcholine and owes its effectiveness in myasthenia gravis to this fact.

Feldberg and Vartiainen (123) have suggested that the persistent excitatory condition in a ganglion following the transmission of an impulse may be due to K. In skeletal muscle Feng et al. (110) have shown that the potentiation of the twitch by a preceding tetanus (post-tetanic enhancement) can be duplicated by the application of K.

Since both K and acetylcholine are formed at nerve endings it is possible that both are formed simultaneously or that one causes the liberation of the other. It is unlikely, however, that acetylcholine could liberate K even though it acts like a base and could exchange

places with K in organic complexes (323). In skeletal muscle certainly the amounts of K are several thousand times as large (in equivalents) as the amounts of acetylcholine liberated so that cation exchange could not account for more than a minute fraction of the total K which escapes during stimulation. Houssay, Marenzi and Gerschman (191) (192) have reported an increase of K in the blood after injection of acetylcholine, but even if true this might be attributed to the muscular twitching which results from the injection.

On the other hand, a liberation of acetylcholine by injection of KCl has been well established in the superior cervical ganglion, the salivary gland, and the tongue (55) (120), in the heart (28) and in the placenta (68). In the placenta it was shown that the effect was due to a transfer of acetylcholine from tissue to blood rather than to a new production of acetylcholine.

If however, potassium represents the normal mechanism for the liberation of acetylcholine then the potassium involved in this process must be scarcely detectable in amount for in skeletal muscle curare abolishes all perceptible loss of K during stimulation (396) (118) without abolishing the appearance of acetylcholine (50) while in the heart during vagal stimulation atropine has the same effect (241). Thus in both heart and skeletal muscle the liberation of K is *largely* the result rather than the cause of the acetylcholine effect. In the skeletal muscle this K is concerned with contraction while in the heart it is apparently concerned with the process of inhibition.

While the excitation of the vagus nerve certainly liberates K in the heart (as has already been mentioned), Wollheim (395) has found that it causes a fall of K and a rise of Ca in the blood of the portal vein after sympathectomy. Opposite effects on K and Ca were produced by sympathetic stimulation after cutting the vagus. The meaning of these facts is not yet clear.

In the salivary gland an increase of K is known to cause a liberation of acetylcholine (120). Brock, Druckrey and Herkin (51) in studying the stimulation of gland slices by acetylcholine or adrenalin (as indicated in respirometers by the resulting increase in O_2 consumption) have shown that only a single stimulation is possible in a K-free medium. Subsequent addition of K permits a repetition of the previous stimulation. This does not prove, however, that K is an essential specific link in the excitation chain for either acetylcholine or adrenalin since any other injurious but reversible change in the solution which rendered the gland non-excitable could have a similar effect. In a later paper, in fact, the

same authors (52) found that a sugar-free medium acted in the same way. It is a striking fact, however, that the glands do not lose their excitability even after prolonged soaking in a K-free medium until after they have been stimulated once.

According to an attractive theory of Zondek (405) (406) the parasympathetic and the sympathetic nervous systems act by changing the K/Ca ratio in the tissues. A relative increase of K is supposed to result from parasympathetic stimulation and to cause its effects and vice versa. While many facts can be reconciled nicely with this theory an equally large number of facts appear to be at variance with it (16) (200) (18). There may be some truth in this theory under special conditions but evidently it is not a universal law, and further difficulties with it will appear in what follows.

Potassium and adrenalin. One of the difficulties with the Zondek theory is the fact that the injection of K (which ought to have only parasympathetic effects) liberates both acetylcholine and adrenalin from the adrenal gland. Presumably the acetylcholine serves to stimulate the gland and to liberate the adrenalin (122). That adrenalin does appear after injection (especially arterial injection) of K and is responsible largely for the resulting pressor effects has been shown in many investigations (230) (158) (18) (195) (208) (169). Conversely the injection of adrenalin, by its effect on the liver, liberates potassium (260) (189) (190) (92) (93) (94) (47). According to Hug (195) the effect of KCl in liberating adrenalin is antagonized by $CaCl_2$ and Kusnetzow (230) perfusing isolated cattle adrenals found a similar inhibition by $CaCl_2$ although Ba and Sr had stimulating effects even more marked than K. Hermann et al. (169) find that NH_4 and Rb and to a lesser extent Li and Cs (but not Na) act like K in this respect. Bacq and Rosenblueth (18) and Katz and Katz (208) find evidence that $CaCl_2$ like KCl liberates adrenaline. This may explain the synergism between Ca and adrenaline which has frequently been reported (264, p. 287).

The effect of K in liberating adrenalin is in part responsible for the rise of blood pressure from arterial injections of KCl which is usually absent from intravenous injections. The pressor effect of intra-arterial KCl is increased by previous injection of adrenalin (266) and vice versa (377). Kylin (231) however, in diabetic patients finds a smaller response to adrenalin after KCl.

Camp and Higgins (62) (63) have demonstrated a widespread parallelism between potassium and adrenalin in their effects upon the blood pressure, heart, etc. They attribute to adrenalin the function of regu-

lating the distribution of K in the body and show that K exhibits its characteristic effects even in the absence of the adrenals. These facts are contrary to the theory of Zondek. In the contraction of the iris, however, the effects of adrenalin and K have been shown to be antagonistic (336). Furthermore the rise of blood pressure resulting from adrenaline is not due to the K which it liberates but may occur equally well with a falling level of blood K (46).

Injected K may therefore cause a rise of blood pressure by a direct peripheral effect or by liberating adrenalin. It may do so also, if the concentration is sufficient, by a central effect (170) (364), and this pressor effect may be duplicated by an injection of K into the floor of the fourth ventricle.

On injection of adrenalin the K first rises and then falls in the blood. The initial rise is so transitory that it can only be detected in man during the first 30–90 seconds after injection, (47) and the rise is greater in arterial then in venous blood because some of the K is removed in passing through the tissues. Because of the transitory nature of this rise, Keys (217) concluded that man differs from the laboratory animals in his response to adrenalin. In spite of this initial rise of K after adrenalin, the subsequent prolonged fall is often a more striking feature of the injection (402) and its cause is unknown unless it represents an over-compensation on the part of the muscles which remove the excess K from the blood (192) (47).

The function of this mobilization of K by adrenalin may possibly be related to muscular exercise which is known to cause the secretion of adrenalin (64). It might be supposed therefore that adrenalin assists in recovery from exercise by returning K from the liver to the muscle. If that is so then adrenalin should cause an increase in the K content of the skeletal muscle. The only direct experimental evidence on this point is apparently the paper of Sugimoto (353) (available to the reviewer in abstract only) who reported an increase in the K content of the quadriceps muscle after adrenalin. In addition adrenalin has been found to inhibit the loss of K and PO_4 from frog muscles immersed in Ringer's solution (and to decrease the electronegativity caused by the application of K to muscle) while parasympathetic drugs had the opposite effect (302). In the small intestine of dogs adrenalin decreases while pilocarpine increases the amounts of K and Mg which can be extracted by trichloracetic acid (104). Finally Dresel and Wollheim (91) in the isolated guinea-pig intestine have reported that adrenalin decreases the K content of the bath while increasing the K content of

the muscle strip, the changes being, however, rather small and irregular. Although these experiments with smooth muscles are not quite pertinent to the question the evidence as a whole may be regarded as possibly suggestive of some rôle played by adrenalin in restoring K to fatigued muscle.

Potassium and asphyxia. Closely related to the adrenalin effect is the effect of asphyxia upon K. Generalized asphyxia of an animal causes a stimulation of the adrenals and a liberation of K (191) (192) (411) (66) (285) (90). Houssay, Marenzi and Gerschman (192) have shown further that the rise of K is dependent chiefly upon the presence of the liver. Presumably therefore adrenalin participates in this reaction.

It is not, however, certain that all the potassium comes from the liver since a loss due to asphyxia has also been observed in the heart (89) (78) and in cat skeletal muscle (20) (119). It appears likely therefore that all tissues tend to give up some K during asphyxia, the greatest release being, however, from the liver as a result of a special adrenalin mechanism. In muscle, however, only a small loss of K was found during circulatory slowing and this loss did not appear to increase with more prolonged stasis (119).

Perhaps the most striking feature of these facts is that the release of K is so small in spite of the large concentration gradient existing between cells and plasma and the large mass of cells surrounding the blood stream. Evidently K is not retained in the cells altogether at the expense of oxidative energy. This is supported by a number of other observations. Frog nerve may be immersed in Ringer's solution in the absence of O_2 for 5 hours without losing more K than is lost under similar conditions in O_2 (111). Likewise in crab nerves Cowan (85) has found no loss of K during temporary asphyxia although prolonged anoxia does have this effect. In frog muscle perfused with an O_2-free solution the loss of K due to anoxia was too small to detect in the perfusate (223). In the frog heart a small initial loss of K was observed in asphyxia (78), but this diminished with time and in any event the loss amounted to less than 3 per cent of the total K present. Red cells retain their K in spite of treatment with oxidative poisons (88). In the plant *Halicystis*, Blinks, Darsie and Snow (31) have shown changes in the membrane potential due to anoxia but have concluded that lack of O_2 decreases the high mobility of K (relative to Na) in the surface, increases the electrical resistance of the protoplasm and protects the cell from loss of its contained electrolytes. Possibly the small

demonstrated loss of K from muscle or other cells in asphyxia is to be regarded as only a secondary effect—a new equilibrium resulting perhaps from an increased concentration of lactic acid or other substances inside the cell. An eventual loss of K after a period of anoxia sufficiently prolonged to kill the cell can hardly be regarded as evidence of a specific connection between oxidation and retention of K.

The high potassium content (29.8 mgm. per cent) of human blood drawn from the heart immediately after death is probably to be regarded as the result of a terminal asphyxia (333). It is possible also that the loss of K from the heart observed by Kehar and Hooker (211) during ventricular fibrillation is likewise the result of diminished coronary flow. If so it is consistent with other observations on asphyxia in that the loss of K was not progressive but tended to disappear as fibrillation continued.

Hemorrhage and shock. As blood is progressively withdrawn from an animal interstitial fluids and later cell fluids move into the vessels to maintain the blood volume and the plasma K concentration increases. For small blood losses the rise of K in the blood is absent or negligible (although there is a K diuresis (352) unaccompanied by a corresponding loss of nitrogen). Kerr (214) found no change of plasma K but a large increase in the K content of the cells (indicating permeability to K). With larger blood losses the plasma K rises more and more steeply as the loss of blood increases (20) (92) (357) (412) (413) (191) (192). Since the increment of lactic acid in the blood is greater (in equivalents) than the increment in K (108) it might be supposed that K came into the blood to neutralize the lactic acid. In spite of this added base, however, Johnston and Wilson (204) have found that the decrease in alkali reserve is greater than can be accounted for by the increment in lactic acid.

Houssay, Marenzi and Gerschman (191) (192) have shown that the K increases in hemorrhage only when the liver is intact. This suggests that the rise of K may be due to the action of adrenalin which is known to be secreted in hemorrhage (53) as it is in asphyxia. The situation in hemorrhage, however, is also similar to that of adrenalectomized animals which lose NaCl and H_2O and must make up the deficiency at the expense of intracellular fluids containing K. For the same reason a rise of plasma K is observed after intestinal fistulas or high intestinal obstruction (30) (334) (335) where the salts contained in the intestinal secretions are not reabsorbed and are therefore continuously lost to the body. Under such conditions the administration of NaCl is bene-

ficial but KCl is toxic (127). In general, K is high also in traumatic shock (413) at least if sufficiently profound (30), in anaphylactic shock (394) (330), in hyperthermic shock (30) and in histamine shock (330) (229) (357). Another similar condition where a rise of K might be expected is heat cramps (355) but according to the data so far available a high K does not appear to be an invariable accompaniment of this malady. An intraperitoneal injection of glucose solution likewise depletes the blood of NaCl and leads generally to a rise of K in the plasma (322). After a rise of K due to hemorrhage an injection of salt solution or of blood causes a return of K to normal (357), and Scudder and Zwemer (334) found the same result with NaCl after an intestinal fistula. This finding supports the theory that the rise of K in hemorrhage represents an attempt to build up the blood volume at the expense of cell fluids after extracellular fluids are exhausted.

The adrenal cortex. Numerous investigations have clearly established the fact that removal of the adrenal cortex leads eventually to low Na and high K concentrations in the serum due to excessive excretion of Na and Cl and defective excretion of K (22) (157) (154) (249) (295) (296) (258). In many respects the symptoms of adrenal insufficiency resemble those of toxic doses of K (414) (415). Potassium is abnormally toxic after adrenalectomy (6) (382), and a low K diet as also a high Na diet is favorable to survival (154) (79). Injection of cortin lowers serum K by decreasing the excretion of Na and increasing the excretion of K (155) (360) (359).

From the point of view of the physiology of potassium it is of prime importance to determine whether the changes in the concentration of this ion which have been described for adrenalectomized animals are directly determined by the presence or absence of cortin or whether cortin acts only on the excretion of Na and Cl, all the other changes being secondary to the resulting decrease of blood volume. A good case can be made out for the latter view for a loss of Na and Cl leads to a loss of water thus simulating the conditions of hemorrhage and shock.

Other observations indicate however that some definite effect of cortin upon potassium both in the kidney and in the tissues has to be postulated in addition. Thus even after removal of the kidneys from adrenalectomized rats the administration of cortin caused the usual fall in the concentration of K in the serum (196). Direct analysis of tissues of adrenalectomized animals for K has provided further evidence. Some investigators have found no change in the potassium content of the muscles in the absence of the adrenal cortex (414) (258), but this may

have been due to an increase in water content of the muscles. Allowing for this factor an increase of K concentration in the intracellular muscle water has been found (160) (150) (287). There is also an increase of K and decrease of Na in the red cells (160), a decrease of K in the liver water (150) and a decrease of K in the liver and the heart (258). Administration of cortin reduces the high muscle potassium to normal (150). A high muscle K might be due to a direct effect upon the muscle potassium equilibrium or to accumulation of excess potassium in the body through failure of the kidney to excrete K, but it could hardly be due merely to failure to excrete Na. There is some evidence therefore that cortin moves K from the muscles to the viscera.

Still further evidence that the adrenal cortex has some specific potassium effect was provided more recently by experiments with desoxycorticosterone (228). After daily administration to dogs for 10 to 20 days the serum K fell to about half the normal value and symptoms resembling those of familial periodic paralysis developed. The paralysis was cured by administration of KCl or withdrawal of the hormone. The relation of K to familial periodic paralysis in human subjects has already been discussed.

Potassium in carbohydrate metabolism. In Addison's disease the blood sugar is usually low and the serum K is high. In other conditions however the sugar and potassium rise and fall together. Thus it has been demonstrated by many investigations that the fall of sugar resulting from insulin is accompanied by a simultaneous fall of K and PO_4 (152) (153) (148) (49) (380) (215) (84) (99) (312) (218). This suggests a formation of hexosephosphate in the muscle and actually the number of equivalents of glucose, K and PO_4 which disappear from the blood are of the same order of magnitude (assuming no simultaneous entrance into the blood). The injection of glucose into the blood of cats and rats lowers K as if sugar were being deposited in tissues in combination with K (124).

This relationship between K and glucose justifies the expectation that K may be high in diabetes untreated with insulin. Unfortunately many reports in the literature leave some doubt concerning the type of treatment employed. It seems probable however that a high serum K is not usually characteristic of this disease (10) (308, p. 787) (318) although some high cases have been reported (314) (348) and both Brems (42) and Kylin (quoted by Brems) have found average values for diabetes slightly higher than normal.

After phlorhizin both sugar and K are low in the blood (206) (403).

Likewise as already mentioned adrenalin liberates both K and sugar from the liver. Snyder and Johnson (346) reported that vagal stimulation tended to cause a decrease in the output of both K and sugar from the perfused liver of the turtle. In many cases, therefore, both K and sugar appear to move into or out of the blood together. One possible reason for this behavior may be that the deposition of glycogen in the liver has been shown to be necessarily accompanied by the deposition of water and potassium (114). The quantity of K so deposited, however, is far less than the quantity of glycogen (measured in equivalents) whereas the blood changes would suggest equivalent amounts. A better explanation is offered by the theory of Conway and Boyle (83) according to which any increase in the indiffusible anions in the cells relative to the diffusible ones would necessitate an intake of both potassium and of water. If therefore inorganic phosphate were combined as hexose-phosphate an intake of K would be predicted.

Since carbohydrate metabolism is also under the influence of the pituitary and hypothalamus, some parallel effects upon K might be expected. The data are not, however, unequivocal on this point. Marenzi and Gerschman (259) found a fall of K after hypophysectomy but no increase in K with injections of anterior lobe extracts, where an increase of sugar is expected. Moreover van Bogaert and van Meel (36) reported a decrease of blood K on stimulation of the hypothalamus of dogs after vagotomy.

Carbohydrate metabolism is clearly influenced by the administration of K although some conflicting facts have been reported. The injection of K into cats and rats causes hyperglycemia and lowers muscle and liver glycogen (341) (342) (343). Similar evidence that high K favors glycogenolysis is seen in the observations of McQuarrie et al. (267) (268) that a high K diet in diabetes increases glycosuria and the excretion of water and decreases the sugar tolerance and the blood pressure while Na has an opposite effect. These facts are in agreement with the effect of K in stimulating the liberation of adrenalin which in turn mobilizes both glucose and K from the liver. In the light of these facts it is difficult to interpret the convincing report of Odashima (301) who found that injection of K *lowers* the blood sugar in rabbits and raises lactic acid. A similar fall of blood sugar after intravenous injection of KCl in man was found by Semler (337) and by Kylin and Engel (232). A hypoglycemia after KCl as well as a hyperglycemia after $CaCl_2$ has also been reported (183) (407).

Just as K and glucose rise and fall together under certain conditions

apparently concerned with movement of glucose into or out of the liver so also there are conditions, related chiefly to muscular activity, under which K and lactic acid appear to move together. Odashima (301) found that K and lactic acid are increased by strychnine and decreased by curare. As already mentioned both substances appear in the venous blood from contracting muscles. An increase in the lactic acid concentration in the blood has been reported in histamine shock (100) along with the increase of K already mentioned. In the salivary gland there is indirect evidence to suggest that K is lost from tissues only when lactic acid is simultaneously escaping (52). In asphyxia and after hemorrhage both K and lactic acid increase in the blood and the equivalent concentration of the lactic acid is more than equal to that of the K. It is tempting to suggest that K leaves the liver or enters muscle in company with glucose while it leaves the muscle or enters the liver along with lactic acid. One difficulty with this hypothesis is that after stimulation K leaves the muscle before lactic acid does (118). Other exceptions to such a general rule must, of course, be anticipated. Some support for such a tentative hypothesis is found in the observation (115) that after muscular activity there is a consistent increase in the K content of the liver. The existence of a K cycle from muscle to liver and other viscera, and back again comparable to the Cori carbohydrate cycle was suggested.

Potassium and nerve function. Like most cells nerves contain K in relatively high concentration inside (117) (331). The effects of various salts on the electrical potential measurements indicate that K has a high mobility in nerves (294) (172). Treatment of nerves with K salts produces characteristic changes in their microscopical appearance, the axis cylinders being broadened and swollen whereas in Ca they are shrunken (179) (254) (105). Similar effects in high concentrations of K and Ca have been reported in muscle (207). Again therefore an increase in the concentration of K outside the cells causes an intake of K and H_2O, i.e., cells swell in KCl.

With sufficient excess of K the oxygen consumption of nerve is depressed (69). The threshold of electrical stimulation is in general first lowered and then raised by K (142) (180) (279) (140) (33) (325). Larger amounts of K, of course, cause inexcitability and in general Ca has an effect opposite to that of K. This modification of excitability by K is to be related to its effect upon the resting membrane potential of the nerve which becomes progressively smaller or increasingly negative outside. Thus the effect of K resembles a cathodal polarization (398)

(140) (96) (233) while the opposite effect is produced by Ca and anodal polarization. According to Lehmann (240) K and alkalinity (or lack of Ca) decrease the negative after potential, increase the positive after potential, lower the threshold and cause spontaneous discharge. Alkalinity acts presumably by decreasing the concentration of Ca ions.

In reflexes or central nervous system transmission K has a similar effect in causing stimulation or increasing excitability. With larger doses there is a depression and the effect of Ca is regularly antagonistic to that of K (186) (187) (161) (351) (133) (364) (37) (319) (169) (286) (106). It has also been found that a small amount of K increases the formation of acetylcholine by brain slices while larger amounts inhibit it (257), thus suggesting a different mechanism in explanation of the K effect.

Elsewhere in the body K also shows excitatory effects. The electrical excitability of human muscle has been found to be increased after ingestion of K salts or alkaline Na salts but not after NaCl or NaH_2PO_4 (300). Intraärterial (282) (283) as well as intravenous (337) injections of potassium are effective in exciting pain receptors in the vessel walls. It has been shown that intradermal injections of K phosphate cause more persistent pain than similar injections of Na phosphate (142 a). It is suggested that pain experienced in inflammation is due to loss of K from neighboring cells. The K content of fluids in such inflammatory areas appears to be large enough to justify this hypothesis. Likewise Brandi (40) has found that local anesthetics or lumbar anesthetics may cause pain or headache if injected with too much K. However, attempts which have been made (221) (222) to demonstrate a high cutaneous irritability when serum K is high do not appear altogether convincing. On the other hand, an excess of K diffusing from injured cutaneous cells has been suggested (109) (177) (326) as cause of the observed adaptation of sensory receptors following trauma to the skin. In accordance with this suggestion Rusk and Kenamore (327) have claimed some success in treating chronic urticaria with a high K diet.

Because of the excitatory effectiveness of K on pain endings and the readiness with which muscles lose K during injury or activity it has been suspected that the muscle pain arising in muscle fatigue, cramps or ischemia might be due to an accumulation of K. Maison (256) however has presented some suggestive evidence against this hypothesis by finding that just as much intramuscularly injected K is required to cause pain after fatigue or ischemia as in a normal muscle. It is possible, of course, that K does not accumulate at a constant rate as

fatigue progresses but appears suddenly at a critical stage. If so, the evidence presented was not decisive.

Potassium and muscle function. Numerous investigators have observed an increase in response, a stimulation, or an increase in excitability of skeletal muscle after administration of small amounts of potassium (59) (227) (277) (19) (159). Careful distinction has not always been made between effects on contractility and effects on excitability but Lapicque and Nattan-Larrier (234) have established a decrease in chronaxie and Chao (71) a decrease in rheobase. Furthermore, in measuring excitability, distinction has not always been drawn between effects on nerve stimulation (which usually causes the response when electrodes are laid on muscles) and the stimulation of muscle proper. Carleton, Blair and Latchford (65), however, have measured the strength-duration curve of the alpha excitability of frog muscle and have found that the administration of K causes an initial decrease of rheobase and increase of the time constant, k, of excitability (or a decrease of chronaxie). Subsequently both quantities change in the reverse direction and the muscle becomes inexcitable.

When applied to the nerve or the end plate, K causes an all-or-none response of the muscle. When applied to the muscle fiber itself in larger concentration, it causes the familiar K contracture (57) or may cause the fiber to give graded responses instead of all-or-none responses to electrical stimuli (349). Similar graded responses with K treatment have been reported in the heart (416). The K contracture is accompanied by phosphocreatine breakdown, lactic acid formation and increased O_2 consumption (159) (290), increased heat production (347) and eventually by inexcitability. According to Solandt (347) the heat production is increased by concentrations of K too small to cause a contracture but Hegnauer, Fenn and Cobb (159) believed the thresholds for the two effects were identical. Later (347) a correlation between contracture and the first phase of heat production was found.

The effects of K on smooth muscle cannot be summarized briefly nor can any such summary be really adequate which does not include at the same time the effects of all the other ions and physico-chemical conditions. The complications of the subject are well illustrated by the recent study of Singh (344) which cannot be briefly summarized. An effect observed after increasing K may often equally well be described as due to relatively too little Ca and vice versa. Since there is an optimum concentration for each ion for a given set of conditions, it is frequently possible to obtain contradictory results over different con-

centration ranges. Thus starting at certain K concentrations a contraction may be produced in a wide variety of smooth muscles by both an increase and a decrease of K (356) (200) (378) (201) (76). Since K may have different effects on frequency, amplitude, tone, etc. an intuitive description of the effects on "activity" is difficult to interpret (225) (371). In general an increase in K/Ca appears to increase tone and decrease frequency (405) (185) (182) (255) (132) (23) (344) or increases the response to pituitrin (362). None of the effects in smooth muscle appear to be fundamentally inconsistent with the initial or low-concentration increase and the subsequent or high-concentration decrease of excitability seen in striated muscle. The increase in tone of smooth muscle is probably comparable to the K contracture of striated muscle.

In heart muscle there are similar difficulties but again, acting for a short time or in small doses, K causes an increase in excitability or decrease of chronaxie (185) (219) (82) and may thus increase the heart rate in the frog (75), the oyster (374) and *Limulus* (70). Even in mammals there is some evidence of a slight initial acceleration of rate (41) (63). In dogs this acceleration has been explained as due to extra-systoles (167) which is not inconsistent with an increase in excitability. The usual decrease in rate observed with K (328) (35) may be due to a central increase in vagal tone (167) or to decrease in excitability (increase in chronaxie) (74). In molluscan hearts in K solutions an increase in tone is observed similar to that in smooth muscle (also in crayfish) (246) and with sufficient K such hearts stop in systole (185) (374). In vertebrate hearts, however, the decrease in excitability with high K solutions has a predominant effect and leads to slowing of the beat, heart block, fibrillation and eventually arrest in distole (262) (193) (379) (391) (291) (358) (149) (295).

When a heart has nearly stopped beating due to lack of K and the K-free solution is then replaced by normal Ringer's solution, the heart at once stops beating completely for a time before it resumes rhythmical contractions. This is the "potassium paradox" of Libbrecht (244) which has been repeatedly confirmed on hearts of various animals (60) (410) (220) (378) (70) (393). The heart having "tried" to compensate for lack of K finds itself overcompensated when a normal K concentration is supplied and has to stop temporarily from an excess K effect until readaptation is complete.

The production of ventricular fibrillation by K may be the result of increased excitability or of local increases in the refractory period.

Excess of K stops this fibrillation (379). This paralyzing property of large doses of K has been utilized in the treatment recommended for the ventricular fibrillation resulting from electric shock (188) (379).

As already mentioned K is low in hearts in cardiac failure. According to Hagen (143) therapeutic doses of digilanid C increase the potassium content of hearts. Toxic doses, however, cause the heart to lose K (375) (143) (397) (67). Cattell and Goodell (67) found a similar mobilization of K from skeletal muscle under the influence of ouabain. According to these results it might be supposed that digitalis and K have somewhat similar effects, and Camp and Higgins (62) have indeed shown that K (as well as epinephrine) potentiates the action of digitalis. Others, however, (406) have postulated a close resemblance between digitalis and Ca.

Potassium in blood. There is some evidence that a larger fraction of the plasma K is indiffusible than is the case with Na. This conclusion has resulted from analyses of plasma and its dialysate for Na and K (141) (324) (197). Quite different evidence of a special association of K with some organic anion was obtained by Waelsch and Kittel (370) who found K migrating to the anode in a special cataphoresis apparatus.

An increase of alkalinity has been found to result from the injection of KCl into the blood (377) or into an isolated heart on a Straub cannula (226). $CaCl_2$ under similar conditions causes an increase in acidity. Of course, changes of this sort in venous blood are very difficult to interpret chiefly because of possible changes in blood flow and respiration. According to Glatzel and Mecke (137), however, intravenous injection of KCl in man increases the pH and lowers the alveolar CO_2 tension and the CO_2 capacity at the arterial point. Probably the extra K is taken up by the cells more readily than the anion leaving an excess of acid in the blood and lowering the alkaline reserve. At the same time the respiration is increased more than enough to compensate for the increased acidity, thus explaining the low alveolar CO_2 and the high pH.

In this connection it is worth noting that K and OH^- usually act synergistically in physiological reactions (48) (240) (132). An increase in OH^- concentration may act by lowering the concentration of diffusible Ca or it might favor the penetration of K as KOH. Komiyama (224) however concludes that in their effects upon the heart K and H^+ act alike, both stopping the heart in diastole, while Ca and OH^- both lead to cessation of beat in systole.

Other potassium changes in blood. Under this heading may be mentioned a rise of arterial K on stimulating the central end of the sciatic nerve (13) (15) which persists after denervation of the liver and after removal of the adrenals, thyroid and pancreas. The writer has several times succeeded in confirming the observation on intact anesthetized cats. The mechanism of the effect is not clear.

A fall of serum K has been seen by many investigators after anesthetics (14) (135) (321) (235) and a rise of K after 2–4 dinitrophenol (235). This has been taken to indicate that the K level in the blood follows the metabolic rate as it often does. It may be doubted, however, whether any one such factor can explain all the vagaries of the K ion.

It is perhaps significant that in many cases an increase in K in the blood plasma is accompanied by a decrease of Ca (390) (408) (386) (293) (156) (107). Odashima (301) has also reported that injection of K salts lowers blood Ca and vice versa. This suggests that both K and Ca are regulated in the blood by the same mechanism. In other conditions, of course, as in various kinds of shock where Na is low Ca may be somewhat elevated as well as K (330) (229). An increase of both K and Ca after ingestion of potassium acetate has also been reported (329).

During epileptic convulsions the plasma K is increased and the Mg is low (176). This especially anti-narcotic or excitatory composition of the blood might be the cause of the seizures but it might also be interpreted as the result of the muscular contractions. McQuarrie (265) however has found an increased excretion of K in the urine just before the convulsions begin.

High plasma K values have also been reported in allergy (394), hypertension and bronchial asthma (42), dermatitis (367) and severe infections and burns (350) and after the injection of NH_4Cl (139) (author unpublished). In the case of NH_4Cl the effect is most reasonably explained as an exchange of K for NH_4^+.

SUMMARY

Potassium is an essential part of the physico-chemical structure of the cell. It is not fixed in position but can move about in the body rather freely according to the demands of shifting membrane equilibria. According to the best evidence at present this is a static equilibrium relatively uninfluenced by lack of oxygen. It probably moves in or out of cells when this equilibrium is disturbed by acid-base imbalances.

It moves *in* when protoplasm grows and *out* when protoplasm disintegrates. It moves from cells to plasma under a variety of conditions all involving an excessive loss of NaCl and water from the body, i.e., hemorrhage, shock, adrenalectomy, intraperitoneal glucose solution, intestinal obstruction or fistulas. K moves into the blood during increased muscular activity or increased rate of metabolism and falls during rest or anesthesia. There is some evidence that it follows the carbohydrate cycle from muscle to liver and back again. It frequently rises and falls with the lactic acid level as in muscular exercise, hemorrhage, and asphyxia; likewise it frequently rises and falls with the sugar level, falling after insulin and rising (temporarily at least) after adrenalin. Possibly it moves from muscle to liver with lactic acid and from liver to muscle with sugar. To some extent potassium is under the control of endocrines being most specifically affected by the secretion of the adrenal cortex. In small concentrations potassium is excitatory and in larger concentrations it is inhibitory. In this capacity it is particularly effective and important in synapses or myoneural junctions. It causes a contracture of skeletal muscle and an increase of tone in heart and smooth muscle under proper conditions. Its effects are usually inhibited by Ca. Since K and Ca modify the most fundamental properties of protoplasm and cells they play a secondary modifying rôle in nearly every vital process. Since the number of ways in which a physiological reaction can be modified are very limited (usually limited to "increase" or "decrease") it is quite possible to classify artificially many drugs, hormones, poisons or other influences as K-like or Ca-like or parasympathetic and sympathetic with more apparent success than is really justified by the complexity of the situation.

REFERENCES

(1) Aronson, H. and P. Sommerfeld. Deutsch. Med. Wchnschr. **39:** 455, 1913.
(2) Adolph, E. F. Am. J. Physiol. **65:** 419, 1923.
(3) Adolph, E. F. Physiol. Rev. **13:** 336, 1933.
(4) Aitken, R. S., E. N. Allott, L. I. M. Castleden and M. Walker. Clin. Sci. **3:** 47, 1937.
(5) Alekseeva, A. M. Bull. Biol. Med. Exp. U.R.S.S. **1:** 303, 1936; Chem. Abs. **32:** 7546, 1938; Physiol. Abs. **22:** 615.
(6) Allers, W. D., H. W. Nilson and E. C. Kendall. Proc. Staff Meet. Mayo Clinic **11:** 283, 1936.
(7) Allott, E. N. and B. McArdle. Clin. Sci. **3:** 229, 1938.
(8) Amberg, S. and H. F. Helmholz. J. Pharmacol. and Exper. Therap. **12:** 19, 1918.
(9) Asada, K. Biochem. Ztschr. **140:** 326, 1923.

(10) ATCHLEY, D. W., R. F. LOEB, E. M. BENEDICT AND W. W. PALMER. Arch. int. Med. **31**: 611, 1923.
(11) AUBERT, H. AND A. DEHN. Pflüger's Arch. **9**: 115, 1874.
(12) AUSTIN, J. H. AND G. D. GAMMEN. J. Clin. Investigation **10**: 287, 1931.
(13) BACHROMEJEW, I. R. Pflüger's Arch. **231**: 426, 442, 449, 1932.
(14) BACHROMEJEW, I. R. AND L. N. PAVLOVA. Arch. Exper. Path. u. Pharmakol. **178**: 390, 1935.
(15) BACHROMEJEW, I. R. AND L. N. PAVLOVA. J. Physiol. (U.S.S.R.) **18**: 69, 1935.
(16) BACQ, Z. M. Ann. de physiol. et. physicochim. biol. **10**: 467, 1934.
(17) BACQ, Z. M. Compt. rend. Soc. de biol. **130**: 1369, 1939.
(18) BACQ, Z. M. AND A. ROSENBLUETH. Am. J. Physiol. **108**: 46, 1934.
(19) BAETJER, A. M. Am. J. Physiol. **109**: 3, 1934.
(20) BAETJER, A. M. Am. J. Physiol. **112**: 139, 1935.
(21) BALL, E. G. J. Biol. Chem. **86**: 449, 1930.
(22) BAUMANN, E. J. AND S. KURLAND. J. Biol. Chem. **71**: 281, 1926.
(23) BAYLISS, L. E., E. BOYLAND AND A. D. RITCHIE. Proc. Roy. Soc. B. **106**: 363, 1930.
(24) DEBEER, E. J., C. G. JOHNSTON AND D. W. WILSON. J. Biol. Chem. **108**: 113, 1935.
(25) BENEDICT, F. G. Data in Sherman (338), p. 272.
(26) BERGAMI, G. Klin. Wchnschr. **15**: 1030, 1936.
(27) DE BETTENCOURT, J. M. AND E. PAES. Arch. portug. Sci. biol. **5**: 133, 1936. Brit. Chem. and Physiol. Abs. AIII, p. 799, 1938. Compt. rend. Soc. de Biol. **122**: 237, 1936.
(28) BEZNÁK, A. B. L. J. Physiol. **82**: 129, 1934.
(29) BINET, L. AND B. MINZ. Arch. internat. physiol. **42**: 281, 1936.
(30) BISGARD, J. D., A. R. MCINTYRE AND W. OSHEROFF. Surgery **4**: 528, 1938.
(31) BLINKS, L. R., M. L. DARSIE, JR. AND R. K. SNOW. J. Gen. Physiol. **22**: 255, 1938.
(32) BLUM, L., E. AUBEL AND R. HAUSKNECHT. Compt. rend. soc. de biol. **85**: 1159, 1921.
(33) BLUMENFELDT, E. Biochem. Ztschr. **156**: 236, 1925.
(34) BOCK, J. Arch. f. exper. Path. u. Pharmakol. **57**: 183, 1907.
(35) BOEHM, R. Arch. f. exper. Path. u. Pharmakol. **75**: 230, 1914.
(36) VAN BOGAERT, A. AND L. VAN MEEL. Compt. rend. Soc. de Biol. **121**: 199, 1936.
(37) BONNET, V. AND F. BREMER. Compt. rend. Soc. de Biol. **126**: 1271, 1937.
(38) BOUCKAERT, J. Arch. int. Physiol. **16**: 453, 1921.
(39) BOURDILLON, J. Am. J. Physiol. **120**: 411, 1937.
(40) BRANDI, B. Ztschr. f. d. ges. exper. Med. **76**: 760, 1931.
(41) BRAUN, L. Pflüger's Arch. **103**: 476, 1904.
(42) BREMS, A. Acta med. scand. (Stockl.) **66**: 473, 1927.
(43) BREWER, A. K. J. Am. Chem. Soc. **59**: 869, 1937.
(44) BREWER, A. K. Science **86**: 198, 1937.
(45) BREWER, A. K. Ind. Eng. Chem. **30**: 892, 1938.
(46) BREWER, G. AND P. S. LARSON. J. Pharmacol. and Exper. Therap. **63**: 272, 1938.

(47) Brewer, G., P. S. Larson and A. R. Schroeder. Am. J. Physiol. **126**: 708, 1939.
(48) Bridges, J. C. and M. Sumwalt. J. Cell and Comp. Physiol. **5**: 199, 1934.
(49) Bridges, A. P., I. Koechig, E. A. Doisy and C. J. Weber. J. Biol. Chem. **58**: 721, 1923.
(50) Brinkman, R. and M. Ruiter. Pflüger's Arch. **204**: 766, 1924.
(51) Brock, N., H. Druckery and H. Herken. Arch. exper. Path. u. Pharmakol. **191**: 687, 1939.
(52) Brock, N., H. Druckery and H. Herken. Biochem. Ztschr. **302**: 393, 1939.
(53) Brooks, C. M. Am. J. Physiol. **114**: 30, 1935.
(54) Brown, G. L. J. Physiol. **91**: 4P, 1937.
(55) Brown, G. L. and W. Feldberg. J. Physiol. **86**: 290, 1936.
(56) Brown, G. L. and F. C. MacIntosh. J. Physiol. **96**: 10, 1939.
(57) Buchthal, F. and J. Lindhard. J. Physiol. **95**: 59P, 1939.
(58) Bureau, V. Arch. internat. de physiol. **45**: 40, 1937.
(59) Burridge, W. J. Physiol. **41**: 285, 1910.
(60) Busquet, H. Compt. rend. Soc. de Biol. **85**: 1142, 1921; **86**: 106, 1922.
(61) Calhoun, J. A., G. E. Cullen, G. Clarke and T. R. Harrison. J. Clin. Investigation **9**: 393, 1930.
(62) Camp, W. J. R. and J. A. Higgins. Science **83**: 622, 1936.
(63) Camp, W. J. R. and J. A. Higgins. Arch. int. de pharmacodyn. et de therap. **61**: 60, 1939.
(64) Cannon, W. B. and S. W. Britton. Am. J. Physiol. **79**: 433, 1927.
(65) Carleton, B. H., H. A. Blair and W. B. Latchford. J. Cell. and Comp. Physiol. **12**: 223, 1938.
(66) Cattell, McK. and H. Civin. J. Biol. Chem. **126**: 633, 1938.
(67) Cattell, McK. and H. Goodell. Science **86**: 106, 1937.
(68) Chang, H. C. Proc. Soc. Exper. Biol. and Med. **34**: 665, 1936.
(69) Chang, T. H., M. Shaffer and R. W. Gerard. Am. J. Physiol. **111**: 681, 1935.
(70) Chao, I. Biol. Bull. **64**: 358, 1933; **66**: 102, 1934.
(71) Chao, I. J. Cell. and Comp. Physiol. **6**: 1, 1935.
(72) Chao, I. Sci. Reports of National Tsing Hua University. Ser. B. **2**: 183, 1937.
(73) Cicardo, V. H. Rev. Soc. argent. Biol. **14**: 12, 1939.
(74) Cicardo, V. H. and A. D. Marenzi. Rev. Soc. argen. Biol. **14**: 59, 1938.
(75) Clark, A. J. J. Physiol. **47**: 66, 1913.
(76) Clark, A. J. J. Pharmacol. and Exper. Therap. **18**: 423, 1921–22.
(77) Clark, A. J. Metabolism of the frog's heart, p. 32, London, 1938.
(78) Clark, A. J., R. Gaddie and C. P. Stewart. J. Physiol. **82**: 265, 1934.
(79) Cleghorn, R. A. and G. A. McVicar. Nature **138**: 124, 1936.
(80) Clowes, J. H. A. and W. S. Frisbie. Am. J. Physiol. **14**: 173, 1905.
(81) Cohn, W. E. and E. T. Cohn. Proc. Soc. Exper. Biol. and Med. **41**: 445, 1939.
(82) Colle, J. Arch. int. de physiol. **29**: 302, 1927.
(83) Conway, E. J. and P. J. Boyle. Nature **144**: 709, 1939.

(84) Cori, C. F. and G. T. Cori. Arch. exper. Path. u. Pharmakol. **172:** 249, 1933.
(85) Cowan, S. L. Proc. Roy. Soc. B. **115:** 216, 1934.
(86) Cuthbertson, D. P. and J. S. Robertson. Proc. Physiol. Soc. **89:** 9P, 1937.
(87) Dale, H. H., W. Feldberg and M. Vogt. J. Physiol. **86:** 353, 1936.
(88) Danielli, J. F. and H. Davson. J. Physiol. **92:** 25P, 1938.
(89) Dennis, J. and R. M. Moore. Am. J. Physiol. **123:** 443, 1938.
(90) Dennis, J. and F. J. Mullin. Proc. Soc. Exper. Biol. and Med. **38:** 560, 1938.
(91) Dresel, K. and E. Wollheim. Pflüger's Arch. **205:** 375, 1924.
(92) D'Silva, J. L. Am. J. Physiol. **82:** 393, 1934.
(93) D'Silva, J. L. J. Physiol. **87:** 181, 1936.
(94) D'Silva, J. L. J. Physiol. **90:** 303, 1937.
(95) Druce, J. G. F. Chem. News **142:** 33, 1931.
(96) Ebbecke, U. Ergebn. Physiol. **35:** 756, 1933.
(97) Eccles, J. C. J. Physiol. **84:** 50P, 1935.
(98) Eichelberger, L. J. Urology **40:** 366, 1928.
(99) Ellsworth, R. and A. Weinstein. Bull. Johns Hopkins Hosp. **53:** 21, 1933.
(100) Eppinger, H., D. Laszlo and A. Schürmeyer. Klin. Wchnschr. **7:** 2231, 1938.
(101) Eppright, E. S. and A. H. Smith. J. Biol. Chem. **118:** 679, 1937.
(102) Epstein, E. Ztschr. f. Krebsforsch. **38:** 63, 535, 1932.
(103) Ernst, E. Naturwiss. **22:** 479, 1934.
(104) Ets, H. N. Am. J. Physiol. **87:** 399, 1928.
(105) Ettisch, G. and J. Jochims. Pflüger's Arch. **215:** 519, 1927.
(106) von Euler, U. S. Skand. Arch. f. Physiol. **80:** 94, 1938.
(107) Ewig, W. and R. Wiener. Ztschr. f. d. g. exper. Med. **61:** 562. 1928
(108) Favata, B. V. and R. H. Koenemann. Unpublished.
(109) Feng, T. P. J. Physiol. **79:** 103, 1933.
(110) Feng, T. P., L. Lee, C. Meng and S. Wang. Chinese J. Physiol. **13:** 79, 1938.
(111) Fenn, W. O. Science Suppl. **79:** 16, 1934.
(112) Fenn, W. O. Physiol. Rev. **16:** 450, 1936.
(113) Fenn, W. O. J. Neurophysiol. **1:** 1, 1938.
(114) Fenn, W. O. J. Biol. Chem. **128:** 297, 1939.
(114a) Fenn, W. O. Am. J. Physiol. **124:** 213, 1939.
(115) Fenn, W. O. Am. J. Physiol. **127:** 356, 1939.
(116) Fenn, W. O. Livro de Homenagem to Professors Alvaro e Miguel and Ozorio de Almeida, p. 197, Rio de Janeiro, Brasil, 1939.
(117) Fenn, W. O., D. M. Cobb, A. H. Hegnauer and B. S. Marsh. Am. J. Physiol. **110:** 74, 1934.
(118) Fenn, W. O., R. H. Koenemann and E. Sheridan. Unpublished.
(119) Fenn, W. O., W. S. Wilde, R. A. Boak and R. H. Koenemann. Am. J. Physiol. **128:** 139, 1939.
(120) Feldberg, W. and J. A. Guimarais. J. Physiol. **86:** 306, 1936.
(121) Feldberg, W. and O. Krayer. Arch. f. exper. Path. u. Pharmakol. **172:** 170, 1933.

(122) FELDBERG, W. AND B. MINZ. Arch. f. exper. Path. u. Pharmakol. **163**: 66, 1932.
(123) FELDBERG, W. AND A. VARTIAINEN. J. Physiol. **83**: 103, 1934.
(124) FLOCK, E., J. L. BOLLMAN, F. C. MANN AND E. C. KENDALL. J. Biol. Chem. **125**: 57, 1938.
(125) FÜHNER, H. Arch. f. exper. Path. u. Pharmakol. **58**: 1, 1907.
(126) GADDUM, J. H., M. A. KHAYYAL AND H. RYDIN. J. Physiol. **89**: 9P, 1937.
(127) GAMBLE, J. L. AND G. S. ROSS. J. Clin. Investigation **1**: 403, 1925.
(128) GAMBLE, J. L., G. S. ROSS AND F. F. TISDALL. J. Biol. Chem. **57**: 633, 1923.
(129) GAMMON, G. D. Proc. Soc. Exper. Biol. and Med. **38**: 922, 1938.
(130) GAMMON, G. D., J. H. AUSTIN, M. D. BLITHE AND C. G. REID. Am. J. Med. Sci. N. S. **197**: 326, 1939.
(131) GAVRILESCU, N. AND N. IONESCU. Compt. rend. Soc. de biol. **124**: 971, 1937.
(132) GELLHORN, E. Pflüger's Arch. **219**: 761, 1928.
(133) GERARD, R. W. AND H. W. MAGOUN. Proc. Soc. Exper. Biol. and Med. **34**: 755, 1936.
(134) GERSCHMAN, R. AND A. D. MARENZI. Compt. rend. Soc. de biol. **120**: 817, 1935.
(135) GERSCHMAN, R. AND A. D. MARENZI. Compt. rend. Soc. de biol. **112**: 509, 1933.
(136) GLATZEL, H. Ztschr. ges. exper. Med. **93**: 666, 1934.
(137) GLATZEL, H. AND W. MECKE. Ztschr. f. d. ges. exper. Med. **91**: 504, 1933.
(138) GLAZKO, A. J. AND D. M. GREENBERG. Am. J. Physiol. **125**: 405, 1939.
(139) GOLLWITZER-MEIER, K. Biochem. Ztschr. **160**: 433, 1925.
(140) GRAHAM, H. T. Am. J. Physiol. **104**: 216, 1933.
(141) GREENE, C. H. AND M. H. POWER. J. Biol. Chem. **91**: 183, 1931.
(142) GREISHEIMER, E. Am. J. Physiol. **49**: 497, 1919.
(142a) HÄBLER, C. AND R. HUMMEL. Klin. Wchnschr. **7**: 2151, 1928.
(143) HAGEN, P. S. J. Pharmacol. and Exper. Therap. **67**: 50, 1939.
(144) HAHN, L., G. HEVESY AND O. REBBE. Nature **143**: 1021, 1939.
(145) HALD, P. T. Arch. f. exper. Path. u. Pharmakol. **53**: 227, 1905.
(146) HAMILTON, J. G. AND G. A. ALLES. Am. J. Physiol. **125**: 410, 1939.
(147) HÄNDEL, M. Biochem. Ztsch. **146**: 420, 1924.
(148) HÄUSLER, H. AND O. HEESCH. Pflüger's Arch. **210**: 545, 1925.
(149) HARRIS, I. AND D. A. LEVIN. J. Physiol. **89**: 153, 1937.
(150) HARRISON, H. E. AND D. C. DARROW. J. Clin. Investigation **17**: 77, 1938.
(151) HARRISON, T. R., C. PILCHER AND G. EWING. J. Clin. Investigation **8**: 325, 1930.
(152) HARROP, G. A. AND E. M. BENEDICT. Proc. Soc. Exper. Biol. and Med. **20**: 430, 1922–23.
(153) HARROP, G. A. AND E. M. BENEDICT. J. Biol. Chem. **59**: 683, 1924.
(154) HARROP, G. A., L. J. SOFFER, R. ELLSWORTH AND J. H. TRESCHER. J. Exper. Med. **58**: 17, 1933.
(155) HARTMAN, F. A., L. LEWIS AND G. TOBY. Science **86**: 129, 1937.
(156) HARTWICH, A. AND G. HESSEL. Klin. Wchnschr. **7**: 67, 1928.
(157) HASTINGS, A. B. AND E. L. COMPERE. Proc. Soc. Exper. Biol. and Med. **28**: 376, 1931.
(158) HAZARD, R. Compt. rend. acad. des sci. **197**: 1455, 1933.

(159) HEGNAUER, A. H., W. O. FENN AND D. M. COBB. J. Cell. and Comp. Physiol. **4:** 505, 1934.
(160) HEGNAUER, A. H. AND E. J. ROBINSON. J. Biol. Chem. **116:** 769, 1936.
(161) HEINEKAMP, W. J. R. J. Pharmacol. and Exper. Therap. **19:** 239, 1922.
(162) HENSAY, J. Pflüger's Arch. **224:** 44, 1930.
(163) HEPPEL, L. Am. J. Physiol. **127:** 385, 1939.
(164) HEPPEL, L. Am. J. Physiol. **128:** 449, 1940.
(165) HEPPEL, L. Am. J. Physiol. **128:** 440, 1940.
(166) HEPPEL, L. A. AND C. L. A. SCHMIDT. Univ. of Calif. Publ. in Physiol. **8:** 189, 1938.
(167) HERING, H. E. Pflüger's Arch. **161:** 544, 1915.
(169) HERMANN, H., F. JOURDAN, G. MORIN AND J. VIAL. Compt. rend. Soc. de Biol. **129:** 593, 595, 1938.
(170) HERMANN, H., F. JOURDAN, G. MORIN AND J. VIAL. Compt. rend. Soc. de Biol. **127:** 315, 1938.
(171) HERRINGTON, M. S. J. A. M. A. **108:** 1339, 1937.
(172) HEUVERSWYN, J. V. Arch. int. de physiol. **43:** 316, 1936.
(173) HILL, S. E. AND W. J. V. OSTERHOUT. J. Gen. Physiol. **17:** 105, 1933.
(174) HILL, S. E. AND W. J. V. OSTERHOUT. J. Gen. Physiol. **22:** 91, 1938.
(175) HILL, S. E. AND W. J. V. OSTERHOUT. J. Gen. Physiol. **22:** 107, 1938.
(176) HIRSCHFELDER, A. D. AND V. G. HAURY. Proc. Soc. Exper. Biol. and Med. **33:** 41, 1935.
(177) HOAGLAND, H. AND M. A. RUBIN. J. Gen. Physiol. **19:** 939, 1936.
(178) HÖBER, R. Pflüger's Arch. **70:** 624, 1898.
(179) HÖBER, R. Zentralb. f. Physiol. **19:** 390, 1905.
(180) HÖBER, R. AND H. STROHE. Pflüger's Arch. **222:** 71, 1929.
(182) HOBSON, A. D. Brit. J. Exper. Biol. **5:** 385, 1928.
(183) HOCHFELD, O. Ztschr. f. d. ges. exper. Med. **37:** 119, 1923.
(184) HOFFMANN, T. Biochem. Ztschr. **243:** 145, 1931.
(185) HOGBEN, L. T. Quart. J. Exper. Physiol. **15:** 263, 1925.
(186) HOOKER, D. R. J. Pharmacol. and Exper. Therap. **4:** 443, 1912.
(187) HOOKER, D. R. Am. J. Physiol. **38:** 200, 1915.
(188) HOOKER, D. R. Am. J. Physiol. **91:** 305, 1929.
(189) HOUSSAY, B. A. AND A. D. MARENZI. Rev. de la Soc. Argent. de Biol. **13:** 139, 1937.
(190) HOUSSAY, B. A. AND A. D. MARENZI. Compt. rend. Soc. de Biol. **126:** 613, 1937.
(191) HOUSSAY, B. A., A. D. MARENZI AND R. GERSCHMAN. Rev. Soc. Arg. Biol. **12:** 434, 1936.
(192) HOUSSAY, B. A., A. D. MARENZI AND R. GERSCHMAN. Compt. rend. Soc. de Biol. **124:** 382, 383, 384, 1937.
(193) HOWELL, W. H. Am. J. Physiol. **15:** 280, 1905.
(194) HOWELL, W. H. AND W. W. DUKE. Am. J. Physiol. **21:** 51, 1908.
(195) HUG, E. Rev. Soc. Argent. Biol. **13:** 483, 1937.
(196) INGLE, D. J., H. W. NILSON AND E. C. KENDALL. Am. J. Physiol. **118:** 302, 1937.
(197) INGRAHAM, R. C., C. LOMBARD AND M. B. VISSCHER. J. Gen. Physiol. **16:** 637, 1932.

(198) INGRAHAM, R. C. AND M. B. VISSCHER. Am. J. Physiol. **114**: 676, 1936.
(199) ISENBERGER, R. M. AND M. W. TYLER. J. Pharmacol. and Exper. Therap. **65**: 461, 1939.
(200) JENDRASSIK, J. Biochem. Ztschr. **148**: 116, 1924.
(201) JENDRASSIK, L. AND E. ANNAU. Biochem. Ztschr. **162**: 207, 1925.
(202) JENDRASSIK, J. AND A. CZIKE. Biochem. Ztschr. **193**: 285, 1928.
(203) JOHNSTON, C. G. AND E. G. BALL. J. Biol. Chem. **86**: 643, 1930.
(204) JOHNSTON, C. G. AND D. W. WILSON. J. Biol. Chem. **85**: 727, 1929.
(205) JOSEPH, M., W. E. COHN AND D. M. GREENBERG. J. Biol. Chem. **128**: 673, 1939.
(206) KASTLER, A. O. J. Biol. Chem. **76**: 643, 1928.
(207) KATO, Y. Folia anatomica Jap. **7**: 293, 1929.
(208) KATZ, G. AND G. KATZ. J. Pharmacol. and Exper. Therap. **59**: 284, 1937.
(209) KAUFMAN, L. AND M. LASKOWSKI. Biochem. Ztschr. **242**: 424, 1931.
(210) KAUNITZ, H. Biochem. Ztschr. **293**: 142, 1937.
(211) KEHAR, N. D. AND D. R. HOOKER. Am. J. Physiol. **112**: 301, 1935.
(212) KENDALL, E. C. AND D. J. INGLE. Science **86**: 18, 1937.
(213) KEITH, N. M. AND M. W. BINGER. J. A. M. A. **105**: 1584, 1935.
(214) KERR, S. E. J. Biol. Chem. **67**: 689, 1926.
(215) KERR, S. E. J. Biol. Chem. **78**: 35, 1928.
(216) KEYS, A. Science **85**: 317, 1937.
(217) KEYS, A. Am. J. Physiol. **121**: 325, 1938.
(218) KEYS, A. Am. J. Physiol. **123**: 608, 1938.
(219) KIRSCH, R. AND H. FREDERICQ. Compt. rend. Soc. de Biol. **103**: 34, 1930.
(220) KISCH, B. Arch. f. exper. Path. u. Pharmakol. **122**: 257, 1927.
(221) KLAUDER, J. V. AND H. BROWN. Arch. Dermat. and Syph. **19**: 52, 1929.
(222) KLAUDER, J. V. AND H. BROWN. Arch. Dermat. and Syph. **20**: 326, 1929; **22**: 877, 1930.
(223) KOENEMANN, R. H., E. T. SHERIDAN AND W. O. FENN. Unpublished.
(224) KOMIYAMA, T. Arch. f. exper. Path. u. Pharmakol. **139**: 100, 1929.
(225) KOCHMANN, M. Biochem. Ztschr. **170**: 230, 1926.
(226) KRAUS, F. AND S. G. ZONDEK. Klin. Wchnschr. **1**: 996, 1922.
(227) KRAUS, F. AND S. G. ZONDEK. Biochem. Ztschr. **156**: 216, 1925.
(228) KUHLMANN, D., C. RAGAN, J. W. FERREBEE, D. W. ATCHLEY AND R. F. LOEB. Science **90**: 496, 1939.
(229) KUSCHINSKY, G. Ztschr. ges. Exper. Med. **64**: 563, 1929.
(230) KUSNETZOW, A. J. Ztschr. ges. Exper. Med. **48**: 671, 1926.
(231) KYLIN, E. Klin. Wchnschr. **4**: 969, 1925.
(232) KYLIN, E. AND A. ENGEL. Klin. Wchnschr. **4**: 653, 1925.
(233) LABES, R. Arch. exper. Path. u. Pharmakol. **168**: 521, 1932.
(234) LAPICQUE, M. AND M. NATTAN-LARRIER. Compt. rend. Soc. de Biol. **94**: 808, 1926.
(235) LARSON, P. S. AND G. BREWER. J. Pharmacol. and Exper. Therap. **67**: 147, 1939.
(236) LASNITZKI, M. Am. J. Cancer **35**: 225, 1939.
(237) LASNITZKI, A. AND M. LASNITZKI. Nature **138**: 800, 1936.
(238) LASNITZKI, A. AND A. K. BREWER. Nature **142**: 538, 1938.
(239) LASNITZKI, A. AND E. A. OESER. J. Chem. Soc., p. 1090, 1937.

(240) LEHMANN, J. E. Am. J. Physiol. **118:** 613, 1937.
(241) LEHNARTZ, E. J. Physiol. **86:** 37P, 1936.
(242) LEITER, L. J. Clin. Investigation **3:** 253, 1926.
(243) LEULIER, A. AND G. VANHEMS. Compt. rend. Soc. de Biol. **115:** 532, 1934.
(244) LIBBRECHT, W. Arch. int. de physiol. **16:** 448, 1921.
(245) LIGHT, A. E., P. K. SMITH, A. H. SMITH AND W. E. ANDERSON. J. Biol. Chem. **107:** 689, 1934.
(246) LINDEMAN, V. F. Physiol. Zool. **1:** 576, 1928.
(247) LIPOW, E. G., C. I. REED AND W. K. WEAVER. Proc. Soc. Exper. Biol. and Med. **28:** 531, 1931.
(248) LISSAK, K. Am. J. Physiol. **126:** P564, 1939.
(249) LOEB, R. F., D. W. ATCHLEY, E. M. BENEDICT AND J. LELAND. J. Exper. Med. **57:** 775, 1933.
(250) LOEWI, O. AND E. NAVRATIL. Pflüger's Arch. **206:** 123, 135, 1924; **214:** 678, 689, 1926.
(251) LORING, F. H. AND J. G. F. DRUCE. Chem. News **140:** 34, 1930.
(252) LOTKA, A. J. Elements of physical biology, p. 197, Baltimore, 1925.
(253) MACALLUM, A. B. Physiol. Rev. **6:** 316, 1926.
(254) MACKUTH, E. Pflüger's Arch. **214:** 612, 1926.
(255) MAGEE, H. E. AND C. REID. J. Physiol. **63:** 97, 1927.
(256) MAISON, G. L. Am. J. Physiol. **127:** 315, 1939.
(257) MANN, P. J. G., M. TENNENBAUM AND J. H. QUASTEL. Biochem. J. **33:** 822, 1939.
(258) MARENZI, A. D. Rev. de la Soc. Arg. de Biol. **14:** 275, 1938.
(259) MARENZI, A. D. AND R. GERSCHMAN. Rev. de la Soc. Arg. Biol. Suppl. to vol. **10:** 350, 1934.
(260) MARENZI, A. D. AND R. GERSCHMAN. Rev. de la Soc. Arg. Biol. **12:** 424, 1936.
(261) MARK, R. E. AND E. KOHL-EGGER. Zentralbl. inn. Med. **48:** 578, 1927.
(262) MARTIN, E. G. Am. J. Physiol. **11:** 370, 1904.
(263) MATHISON, G. C. J. Physiol. **42:** 471, 1911.
(264) MCDOWALL, R. J. S. The control of circulation of the blood. London, 1938.
(265) MCQUARRIE, I. Ann. Int. Med. **6:** 497, 1932.
(266) MCGUIGAN, H. A. AND J. A. HIGGINS. Am. J. Physiol. **114:** 207, 1935.
(267) MCQUARRIE, I. AND W. H. THOMPSON. Proc. Soc. Exper. Biol. and Med. **31:** 907, 1934.
(268) MCQUARRIE, I, W. H. THOMPSON AND J. A. ANDERSON. J. Nutrition **11:** 77, 1936.
(269) MENDEL, B., D. MUNDELL AND F. STRELITZ. Nature **144:** 479, 1939.
(270) MENTEN, M. L. J. Med. Res. **43:** 323, 1922.
(271) MEYER-DÖRKEN, G. Beitr. z. path. Anat. und zur allg. Path. **83:** 747, 1930.
(272) MICHAELIS, L. J. Gen. Physiol. **8:** 33, 1925.
(273) MILLER, H. G. J. Biol. Chem. **67:** 71, 1926.
(274) MILLER, H. G. J. Biol. Chem. **55:** 61, 1923.
(275) MILLER, H. G. J. Biol. Chem. **70:** 587, 1926.
(276) MILLER, H. G. AND W. W. YATES. J. Biol. Chem. **62:** 259, 1924.
(277) MINES, G. R. J. Physiol. **37:** 408, 1908.

(278) Minot, A. S. J. Path. and Exper. Therap. **66:** 453, 1939.
(279) Misske, B. Biochem. Ztschr. **219:** 320, 1930.
(280) Mitchell, J. K., S. Flexner and D. L. Edsall. Brain **25:** 109, 1902.
(281) Mond, R. and H. Netter. Pflüger's Arch. **230:** 42, 1932.
(282) Moore, R. M. Am. J. Physiol. **110:** 191, 1934.
(283) Moore, R. M., R. E. Moore and A. O. Singleton, Jr. Am. J. Physiol. **107:** 594, 1934.
(284) Morávek, V. Biochem. Ztschr. **258:** 340, 1933.
(285) Mullin, F. J., J. Dennis and D. B. Calvin. Am. J. Physiol. **124:** 192, 1938.
(286) Mullin, F. J., A. B. Hastings and W. M. Lees. Am. J. Physiol. **121:** 719, 1938.
(287) Muntwyler, E., F. R. Mantz, G. Mangun and R. C. Mellors. J. Biol. Chem. **287:** 128, lxxiv, 1939.
(288) v. Muralt, A. Verh. Freien Vereinigung Schweizerischer Physiologen—12th meeting Lausaune, June, 1937, p. 17.
(289) Myers, V. C. and J. J. Short. J. Biol. Chem. **48:** 83, 1921.
(290) Nachmansohn, D., J. Wajzer and A. Marnay. Compt. rend. Soc. de Biol. **121:** 141, 1936.
(291) Nahum, L. H. and H. E. Hoff. J. Pharmacol. and Exper. Therap. **65:** 322, 1939.
(292) Nakashima, K. Pflüger's Arch. **158:** 288, 1914.
(293) Nelken, L. and H. Steinitz. Ztschr. klin. Med. **103:** 317, 1926.
(294) Netter, H. Pflüger's Arch. **218:** 310, 1928.
(295) Nicholson, W. M. and L. J. Soffer. Johns Hopkins Hosp. Bull. **56:** 236, 1935.
(296) Nilson, H. W. Am. J. Physiol. **118:** 620, 1937.
(297) Noguchi, I. Arch. f. exper. Path. u. Pharmakol. **108:** 73, 1925.
(298) Noonan, T. R., L. Haege and W. O. Fenn. Proc. Am. Physiol. Soc., New Orleans, 1940.
(299) Norn, M. Skand. Arch. f. Physiol. **55:** 184, 1929.
(300) Nothmann, M. and A. Wagner. Arch. f. exper. Path. u. Pharmakol. **101:** 17, 1924.
(301) Odashima, G. Tohoku J. Exper. Med. **18:** 250, 1932.
(302) Okamoto, Y. Pflüger's Arch. **204:** 726, 1924.
(303) Olmer, D., L. Payan and J. Berthier. Compt. rend Soc. de Biol. **87:** 867, 1922; **89:** 330, 1923.
(304) Osborne, E. D. J. A. M. A. **79:** 615, 1922.
(305) Osborne, T. B. and L. B. Mendel. J. Biol. Chem. **34:** 131, 1918.
(306) Osterhout, W. J. V. and S. E. Hill. J. Gen. Physiol. **17:** 105, 1933.
(307) Osterhout, W. J. V. and S. E. Hill. Proc. Soc. Exper. Biol. and Med. **32:** 715, 1935.
(308) Peters, J. P. and D. D. Van Slyke. Quantitative clinical chemistry. Vol. 1. Baltimore, 1932.
(309) Pichler, Z. Arch. Exper. Path. u. Pharmakol. **175:** 85, 1934.
(310) Pohlmann, J. Pflüger's Arch. **240:** 377, 1938.
(311) Pohlmann, J. and H. Netter. Naturwiss. **26:** 138, 1938.
(312) Posatti, F. and W. Beiglblöck. Mschr. Geburtsh. Gynäk. **108:** 237, 1938.
(313) Rabinowitch, I. M. J. Biol. Chem. **62:** 667, 1924.

(314) Rathery, F. and J. Bertoliatti. Compt. rend. Soc. de Biol. **116:** 1346, 1934.
(315) Redina, L. Biochem. Ztschr. **177:** 253, 1926.
(316) Reginster, A. Arch. int. Physiol. **45:** 69, 1937; **67:** 71, 1938.
(317) Reinhold, J. G. and D. W. Wilson. Am. J. Physiol. **107:** 378, 1934.
(318) Remond, A. and E. Cantegril. Compt. rend. Soc. de Biol. **103:** 63, 1930.
(319) Resnik, H., M. F. Mason, R. T. Terry, C. Pilcher and T. R. Harrison. Am. J. Med. Sci. (N.S.) **191:** 835, 1936.
(320) Rittmann, R. and O. Form. Ztschr. f. d. ges. Exper. Med. **48:** 191, 1925.
(321) Robbins, B. H. and H. A. Pratt. J. Pharmacol. and Exper. Therap. **56:** 205, 1936.
(322) Robinson, E. J. and A. H. Hegnauer. J. Biol. Chem. **116:** 779, 1936.
(323) Roepke, M. H. and A. deM. Welsh. J. Pharmacol. and Exper. Therap. **56:** 319, 1936.
(324) Rona, P. and H. Petow. Biochem. Ztschr. **137:** 356, 1923.
(325) Rosenberg, H. and K. Kitayama. Pflüger's Arch. **223:** 602, 1929.
(326) Rubin, M. A. and B. J. Syrocki. J. Cell. Comp. Physiol. **9:** 29, 1936.
(327) Rusk, H. A. and B. D. Kenamore. Ann. int. Med. **11:** 1838, 1938.
(328) Sakai, T. Ztschr. f. Biol. **64:** 505, 1914.
(329) Sauer, J. Deutsch. Arch. f. Klin. Med. **172:** 223, 1931.
(330) Schittenhelm, A., W. Erhardt and K. Warnat. Ztschr. ges. exper. Med. **58:** 662, 1928.
(331) Schmitt, F. O. and R. S. Bear. Am. J. Physiol. **126:** P621, 1939.
(332) Schwarz, H. Arch. f. exper. Path. u. Pharmakol. **177:** 628, 1935.
(333) Scudder, J., M. E. Smith and C. R. Drew. Am. J. Physiol. **126:** 337, 1939.
(334) Scudder, J. and R. L. Zwemer. Surgery **2:** 519, 1937.
(335) Scudder, J., R. L. Zwemer and R. Truszkowski. Surgery **1:** 74, 1937.
(336) Seager, L. D. J. Pharmacol. and Exper. Therap. **66:** 202, 1939.
(337) Semler, R. Klin. Wchnschr. **4:** 697, 1925.
(338) Sherman, H. C. Chemistry of food and nutrition. 2nd ed. Macmillan, New York, 1918.
(339) Shinosaki, T. Ztschr. ges. Neurol. u. Psychiat. **100:** 564, 1926.
(340) Shohl, A. T. and A. Sato. J. Biol. Chem. **58:** 235, 1923.
(341) Silvette, H. Am. J. Physiol. **123:** P. 187, 1938.
(342) Silvette, H. and S. W. Britton. Proc. Soc. Exper. Biol. and Med. **37:** 252, 1937.
(343) Silvette, H., S. W. Britton and R. Kline. Am. J. Physiol. **122:** 524, 1938.
(344) Singh, I. J. Physiol. **96:** 367, 1939.
(345) Smith, A. H. and P. K. Smith. J. Biol. Chem. **107:** 681, 1934.
(346) Snyder, C. D. and R. E. Johnson. Johns Hopkins Hosp. Bull. **62:** 110, 1938.
(347) Solandt, D. Y. J. Physiol. **86:** 162, 1936; also ibid., **93:** 305, 1938.
(348) Staub, H., Fr. Günther and R. Fröhlich. Klin. Wchnschr. **2:** 2337, 1923.
(349) Steiman, S. E. Am. J. Physiol. **118:** 492, 1937.
(350) Stenger, K. Klin. Wchnschr. **18:** 576, 1939.
(351) Stern, L., J. A. Rossine and G. J. Chvoles. Compt. rend. Soc. de Biol. **114:** 674, 1933.

(352) Stewart, J. D. and G. M. Rourke. J. Clin. Investigation **15**: 697, 1936.
(353) Sugimoto, S. Folia Endocrinol. Japon **8**: 23, 1932. See Chem. Abs. **27**: 5824, 1933.
(354) Szent-Györgyi, A., Z. M. Bacq and M. Goffart. Nature **143**: 522, 1939.
(355) Talbot, J. H. Medicine **14**: 323, 1935.
(356) Tate, G. and A. J. Clark. Arch. int. Pharm. Therap. **26**: 103, 1922.
(357) Thaler, J. I. Proc. Soc. Exper. Biol. and Med. **33**: 368, 1935.
(358) Thomson, W. A. R. Lancet **236**: 808, 1939.
(359) Thorn, G. W., H. R. Garbutt, F. A. Hitchcock and F. A. Hartman. Proc. Soc. Exper. Biol. and Med., **35**: 247, 1936.
(360) Thorn, G. W., R. P. Howard and K. Emerson, Jr. J. Clin. Investigation **18**: 449, 1939.
(361) Tipton, S. R. Am. J. Physiol. **124**: 322, 1938.
(362) Van Dyke, H. B. and A. B. Hastings. Am. J. Physiol. **83**: 563, 1928.
(363) Vineberg, A. M. and S. A. Komarov. Am. J. Physiol. **104**: 73, 1933.
(364) Vleeschouver, G. de. Arch. int. pharm. et therap. **50**: 251, 1935. Quoted by Hermann et al. (169).
(365) Vogt, M. J. Physiol. **86**: 258, 1936.
(366) Voit, C. Die Ernahrung, Vol. 6, 362. Handbuch der Physiol. L. Hermann, Leipzig, 1881.
(367) Umansky, G. I. and A. P. Stepanowa. Wien. klin. Wchnschr. **46**: 1262, 1933.
(368) Wach, E. C. J. Dental Res. **15**: 265, 1936.
(369) Wachholder, K. and Fr. Matthias. Pflüger's Arch. **232**: 159, 1933.
(370) Waelsch, H. and S. Kittel. Kolloidztschr. **66**: 200, 1934.
(371) Wagner, W. Biochem. Ztschr. **172**: 149, 1926.
(372) Walker, M. B. Lancet **226**: 1200, 1934; **229**: 47, 1935.
(373) Wallace, G. B. and A. R. Cushny. Am. J. Physiol. **1**: 411, 1898.
(374) Walzl, E. M. Physiol. Zoöl. **10**: 125, 1937.
(375) Wedd, A. M. J. Pharmacol. and Exper. Therap. **65**: 268, 1939.
(376) Weiler, G. J. Physiol. **96**: 51P, 1939.
(377) Weiss, St. and B. Dunay. Ztschr. f. d. ges. Exper. Med. **59**: 474, 1928.
(378) Wells, G. P. Brit. J. Exper. Biol. **5**: 258, 1928.
(379) Wiggers, C. J. Am. J. Physiol. **93**: 197, 1930.
(380) Wigglesworth, V. E., C. E. Woodrow, W. Smith and L. B. Winter. J. Physiol. **57**: 447, 1922.
(381) Wilde, W. S. J. Biol. Chem. **128**: 309, 1939.
(382) Wilder, R. M., E. C. Kendall, A. M. Snell, E. J. Kepler, E. H. Rynearson and M. Adams. Arch. Int. Med. **59**: 367, 1937.
(383) Wiley, F. H. and L. L. Wiley. J. Biol. Chem. **101**: 83, 1933.
(384) Wiley, F. H., L. L. Wiley and D. S. Waller. J. Biol. Chem. **97**: lvi, 1932.
(385) Wiley, F. H., L. L. Wiley and D. S. Waller. J. Biol. Chem. **101**: 73, 1933.
(386) Wilkins, L. and B. Kramer. Arch. Int. Med. **31**: 916, 1923.
(387) Wilkins, W. E. and G. E. Cullen. J. Clin. Investigation **12**: 1063, 1933.
(388) Wills, J. H. and W. O. Fenn. Am. J. Physiol. **124**: 72, 1938.
(389) Wilson, A. T. and S. Wright. Quart. J. Exper. Physiol. **26**: 127, 1936.
(390) Winkler, H. Monatschr. f. Geburtsh. u. Gynäk. **100**: 223, 1935; **101**: 34, 1935.

(391) WINKLER, A. W., H. E. HOFF AND P. K. SMITH. Am. J. Physiol. **124:** 478, 1938; **127:** 430, 1939.
(392) WINKLER, A. W. AND P. K. SMITH. J. Biol. Chem. **124:** 589, 1938.
(393) WITANOWSKI, W. R. Pflüger's Arch. **212:** 726, 1926.
(394) WOLFRAM, J. AND R. L. ZWEMER. J. Exper. Med. **61:** 9, 1935.
(395) WOLLHEIM, E. Biochem. Ztschr. **151:** 416, 1924.
(396) WOOD, E. H., D. A. COLLINS AND G. K. MOE. Proc. Am. Physiol. Soc. **126:** P657, 1939.
(397) WOOD, E. H. AND G. K. MOE. Am. J. Physiol. **123:** 219, 1938.
(398) WORONZOW, D. S. Pflüger's Arch. **203:** 300, 1924.
(399) YAGI, S. Arch. int. Pharm. **22:** 259, 1912.
(400) YOSHIMURA, K. Ztschr. f. d. ges. Exper. Med. **70:** 251, 1930.
(401) YOUNG, A. C. J. Neurophysiol. **1:** 4, 1938.
(402) ZAMORANI, V. Riv. di Clin. Pediat. **26:** 288, 1928.
(403) ZIEGLER, M. R. AND I. MCQUARRIE. Proc. Soc. Exper. Biol. and Med. **39:** 142, 1938.
(404) ZIELSTORFF, W. Handbuch d. n. u. path. physiol. xvi/2, 1674, Berlin, 1931.
(405) ZONDEK, S. G. Biochem. Ztschr. **132:** 362, 1922.
(406) ZONDEK, S. G. Die Elektrolyte, p. 122, Berlin, 1927.
(407) ZONDEK, S. G. AND A. BENATT. Ztschr. ges. Exper. Med. **43:** 281, 1924.
(408) ZONDEK, H., H. PETOW AND W. SIEBERT. Klin. Wchnschr. **1:** 2172, 1922.
(409) ZONDEK, H., H. PETOW AND W. SIEBERT. Ztschr. f. klin. Med. **99:** 129, 1923.
(410) ZWAARDEMAKER, H. J. Physiol. **53:** 273, 1919.
Klin. Wchnschr. **1:** 535, 594, 1922.
(411) ZWEMER, R. L. AND F. H. PIKE. Ann. N. Y. Acad. Sci. **37:** 257, 1938.
(412) ZWEMER, R. L. AND J. SCUDDER. Am. J. Physiol. **119:** 427, 1937.
(413) ZWEMER, R. L. AND J. SCUDDER. Surgery **4:** 510, 1938.
(414) ZWEMER, R. L. AND R. TRUSZKOWSKI. Science **83:** 558, 1936.
(415) ZWEMER, R. L. AND R. TRUSZKOWSKI. Proc. Soc. Exper. Biol. and Med. **35:** 424, 1936.
(416) ZWIKSTER, G. H. AND T. E. BOYD. Am. J. Physiol. **113:** 560, 1935.

OTHER REVIEWS

HEUBNER, W. Mineralbestand des Körpers Handbuch d. norm. u. path. Physiol. xvi/2, pp. 1416–1516. Berlin, 1931.
KLINKE, K. Der Mineralstoffwechsel. Physiol. u. Path. Leipzig and Vienna, Franz Deutiche, 1931. In K. K. Barrenscheen et al. Einzeldarstellungen aus dem Gesamtgebiet der Biochem. Bd. 3.
MEYER-BISCH, R. Umsatz der Alkalichloride. Handbuch d. norm. u. Path. Physiol. xvi/2, pp. 1517–1554. Berlin, 1931.
SHOHL, A. T. Annual Rev. Biochemistry **3:** 209, 1934.
WEBSTER, R. W. AND W. A. BRENNAN. Potassium and tartrates. Chicago, 1927.
WENDT, G. v. Oppenheimer's Handbuch d. Biochem. **8:** 183, 1925.
See also references no. 308 and 112.

THE PORPHYRINS IN HEALTH AND DISEASE

KONRAD DOBRINER AND C. P. RHOADS

The Memorial Hospital, New York City and The Hospital of The Rockefeller Institute for Medical Research, New York City

Few subjects have been the source of so much confusion as that of the porphyrins. Furthermore, few have been of such profound importance to medicine, since an understanding of the metabolism of the pyrrol compounds is essential to any clear conception of the normal or pathological physiology of the respiratory pigments, and these substances make up one of the most important systems of the body. Two sources of confusion have existed in the past: the complicated nomenclature applied to the porphyrins, and a lack of understanding by clinicians of the essential procedures for studying their metabolism. In view of this fact, whereas by convention it is proper in a review article to present all sides of a subject and to give impartial consideration to conflicting results of experimental work, this has not always been done in this communication. Any omissions, however, are due solely to a desire to simplify the presentation, and to the fact that many reports must be discarded because they lack accurate information concerning the types, kinds, and amounts, of porphyrins excreted. In an attempt to attain clarity, therefore, a general survey is presented, so simplified that it should be useful to those who have not been particularly familiar with the problems of pyrrol chemistry.

The chemistry of the porphyrins. Hemoglobin is a complex made up of a pigment of the pyrrol group known as protoporphyrin, combined with iron, and with a protein, globin. A chemically related, but artificial, product of hemoglobin disintegration, hematoporphyrin, was first produced in its crystalline form by Nencki and Sieber (111). It became apparent from the structure of this compound (Piloty, Küster, Willstätter and Fischer, 34) that it was not the porphyrin present in hemoglobin (protoporphyrin); that is, the pigment which would remain behind if iron and globin were removed. Kämmerer (72a) in 1924 however, isolated a porphyrin formed from blood by bacterial action. The work was continued by Fischer (34a), who with Schneller (34f)

and Lindner (34k) in 1925 identified Kämmerer's pigment as a protoporphyrin. Fischer and Zeile (35h), and Fischer, Treibs, and Zeile (35i) synthesized protoporphyrin in 1929 from simple pyrrols. They found that the synthetic iron-protoporphyrin compound was identical with the natural heme.

Salkowski (125), Garrod (41b), Stockvis (139) and others found porphyrins excreted in the urine in certain diseases. Garrod (41b) as well as Saillet (124), demonstrated the presence of small amounts of similar pigments in normal urines. They supposed that they had isolated hematoporphyrin since until 1915 investigators were unable to distinguish artificial porphyrins from the natural compounds. Prior to Fischer's work the term hematoporphyrin was used to designate any porphyrin excreted in the urine.

In 1915 Hans Fischer, working as a young chemist in the laboratory of Friedrich von Müller in Munich, was engaged in a study of the nature of the pigments in blood and bile. A patient named Petry, who has since become famous as the classical case of congenital porphyria, excreted large amounts of unidentified pigments. These were supposed to be responsible for the necrosis of his skin which occurred after exposure to sunlight. Fischer isolated two different pigments from the excreta of this case (34a, b, c). One of them, which he termed coproporphyrin, was found in both urine and feces, and the other, chemically different, called uroporphyrin, was found only in the urine. It is now known that small amounts of the same coproporphyrin are found in normal urine and feces, but that the uroporphyrin is a pathological product excreted almost exclusively in congenital porphyria.

This observation was the beginning of a series of studies of the relationship of the excreted porphyrins to the respiratory pigments, and to the products of their destruction. The type of uroporphyrin and coproporphyrin excreted by normals and in congenital porphyria was found to be fundamentally different from that of the protoporphyrin in hemoglobin. Later it was shown that two types of porphyrins are present in yeast also (34n). This simultaneous existence of two chemically distinct types of compounds was termed by Fischer the "dualism of the porphyrins" (34).

To understand this dualism the chemistry of the porphyrins must be considered briefly (34). The basic structure consists of 4 pyrrol rings linked together by 4 methene bridges, and is the porphin nucleus present in all porphyrins. It was synthesized in 1935 by Fischer and Gleim (35o), and independently by Rothemund (122) (fig. 1).

The hydrogen atoms numbered 1 to 8 in the formula (fig. 1) are replaced in the naturally occurring porphyrins by a series of substituting groups. For example, the protoporphyrin (fig. 2) present in hemoglobin and myoglobin has 4 methyl (1, 3, 5, 8), 2 propionyl (6, 7), and 2 vinyl (2, 4), groups substituted in place of the 8 hydrogen atoms in the basic porphin nucleus.

If the 8 hydrogen atoms are replaced by 4 methyl and 4 ethyl groups, 4 isomeric compounds can be derived, termed etioporphyrins types I, II, III and IV. They do not exist in nature, but are the basic structures to which the porphyrins are referred for purposes of identification. All the naturally occurring porphyrins so far analyzed have been found to correspond to etioporphyrins types I and III (fig. 3).

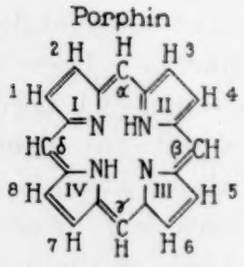

Fig. 1

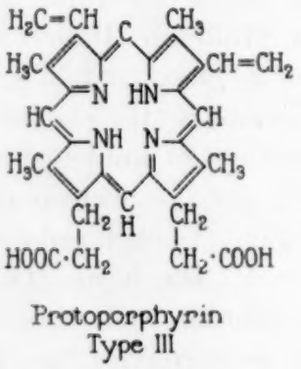

Fig. 2

Fig. 1. The structural formula of porphin
Fig. 2. The structural formula of protoporphyrin type III

If the 8 hydrogen atoms are replaced by 3 types of substituting groups, namely: 4 methyl, 2 propionyl, and 2 vinyl, compounds are obtained which are called protoporphyrins. Fifteen isomeric porphyrins of this kind are theoretically possible. The porphyrin in both hemoglobin and myoglobin, as well as the prosthetic group of other respiratory enzymes is protoporphyrin.

The vinyl groups of the protoporphyrins are transformed by mild reduction into ethyl groups, and in this way porphyrins are obtained which are called mesoporphyrins. For the 8 hydrogen atoms present in the porphin, 4 methyl, 2 ethyl, and 2 propionyl groups are substituted. Fifteen isomeric mesoporphyrins are theoretically possible. Mesoporphyrin was recently found in the feces by Zeile and Rau (161a). Fischer (34d) has reported the transformation of the protoporphyrin

present in natural heme into mesoporphyrin. Two different types of mesoporphyrin were isolated.

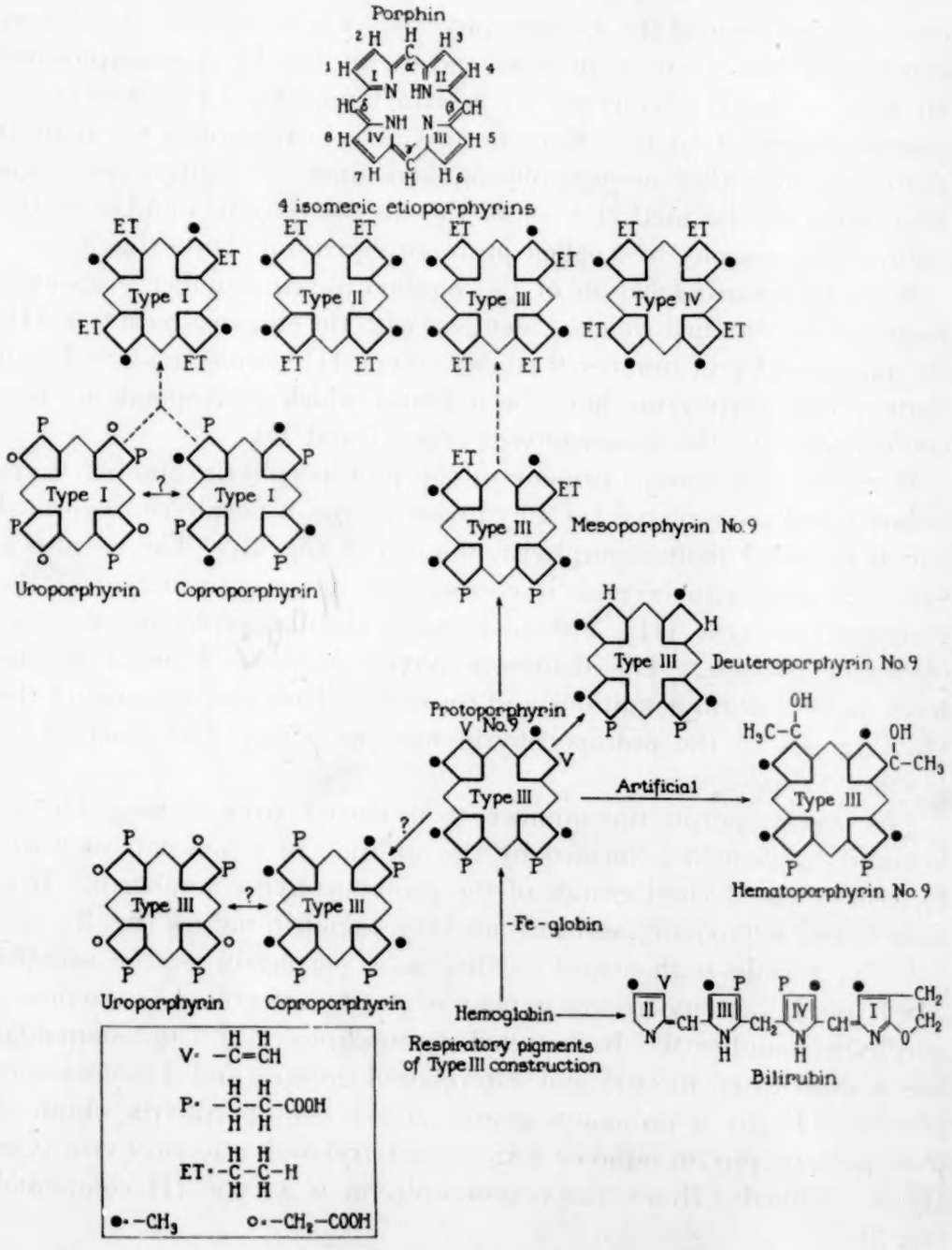

Fig. 3. The structural formulae of the porphyrins

If the 2 carboxyl groups in the mesoporphyrins are decarboxylated, etioporphyrins are produced which have 4 methyl and 4 ethyl groups

in place of the 8 hydrogen atoms. Only 4 isomers of etioporphyrin are possible, however (fig. 3).

Each of the 15 isomers of protoporphyrin and of mesoporphyrin corresponds to one of the 4 etioporphyrins. Fischer and his co-workers synthesized the 4 etioporphyrins and 12 of the 15 mesoporphyrins. He gave to the etioporphyrins the arbitrary numbers 1 to 4, and to the mesoporphyrins 1 to 15. Since he was able to transform the natural protoporphyrin into mesoporphyrin, and since its methyl ester was identical with the methyl ester of the mesoporphyrin number 9, the natural protoporphyrin is called protoporphyrin number 9 (fig. 3).

Since by decarboxylation of the mesoporphyrin number 9 an etioporphyrin is obtained which is identical with the etioporphyrin type III, the protoporphyrin number 9 is also a type III porphyrin. So far in nature only porphyrins have been found which correspond in their configuration to the etioporphyrin types I and III.

If the 2 vinyl groups present in the protoporphyrin number 9 are reduced and are replaced by 2 hydrogen atoms, a porphyrin is formed which is called deuteroporphyrin number 9 (fig. 3). This is also a type III porphyrin because it corresponds in its construction to the etioporphyrin type III. Fifteen isomeric deuteroporphyrins are theoretically possible. The deuteroporphyrin number 9 found in the feces under certain conditions and formed by bacterial removal of the vinyl groups of the protoporphyrin number 9 was first isolated by Watson (156a).

The hematoporphyrin number 9 produced from hemoglobin or hematin by Nencki is formed by the addition of 1 molecule of water to each of the 2 vinyl groups of the protoporphyrin number 9. It is an artificial compound, and has not been found in nature (fig. 3).

Under certain pathological conditions, a porphyrin can be isolated which has 2 propionyl groups in place of the 2 vinyl groups of the protoporphyrin number 9. It is called coproporphyrin. This compound has 4 substituted methyl and 4 propionyl groups, and 4 isomers are possible. If the 4 propionyl groups of the coproporphyrin obtained from protoporphyrin number 9 are decarboxylated, etioporphyrin type III is obtained. Hence the coproporphyrin is a type III compound (fig. 3).

The transformation of protoporphyrin number 9 into coproporphyrin type III may take place in nature but has not been effected in vitro. Coproporphyrin III was isolated first from a case of chronic porphyria by Hijmans v. d. Bergh and co-workers (61a), and was finally identified by Fischer and his associates (35f).

If the 4 methyl groups of the coproporphyrin type III are carboxylated a compound is formed which is called uroporphyrin type III. This compound was obtained first by Waldenström (155a) from the urine of a patient with acute porphyria. By decarboxylation uroporphyrin type III can be transformed into coproporphyrin type III (fig. 3).

The protoporphyrin number 9, the deuteroporphyrin number 9, the mesoporphyrin number 9, the coproporphyrin type III, and the uroporphyrin type III, are chemically closely related compounds. Hence a transformation of one into the other easily can be understood (fig. 3).

Fischer and his staff, Warburg and Negelein, Zeile, Stern, Schönheimer and others investigated the configuration of the porphyrins in the respiratory pigments (34). They proved that hemoglobin, (34, 35h, i), myoglobin (127), cytochrome C (161b), catalase (138), spyrographis-hemin (35g), and other pigments all contain porphyrins of type III configuration. Types other than III were not found in the respiratory pigments until the recent discovery by Fischer (34d) of a type I porphyrin in natural heme.

In 1929 Fischer (34, 35h, i) synthesized heme from simple pyrrols, which he combined into suitably constructed pyrromethenes. These were then condensed into a type III porphyrin and by further steps transformed into protoporphyrin number 9 (type III). By the combination of the latter with iron he obtained heme, which showed all the properties of the natural substance.

The exact steps of the natural synthesis are unknown, but it is assumed that it starts with very simple building stones. Fischer has suggested that possibly aceto-acetic acid may be one of the sources, and others have suggested proline, oxyproline, or tryptophane.

In the physiologic breakdown of hemoglobin bilirubin is formed (fig. 3), and is transformed by further reduction and oxidation into urobilin and urobilinogen, as well as into other bile pigments (34, 156). In the formation of bilirubin the porphin ring is opened in the alpha position and a chain compound results with a structure corresponding to type III porphyrin. Recent investigations (Lemberg, 83a, b, c; Barkan, 4a, b) indicate that under normal conditions the porphyrin ring is opened while it contains iron and while it is still combined with the protein molecule. It is important to note that in the normal transformation of hemoglobin into bile pigment no free porphyrin occurs.

Fischer isolated in 1915 from the feces of Petry a porphyrin which he called coproporphyrin because of its source, although it was later found in the urine also (34, 34a, b, c). He established the fact that this porphyrin is fundamentally different in its type from protoporphyrin

number 9 (type III) present in hemoglobin. As shown in figure 3, the coproporphyrin is similar in configuration to the etioporphyrin type I, and hence is a type I porphyrin. This compound was isolated from the urine and feces of normal human beings (Hoerburger, 64a; Watson, 156e; Dobriner, 23b; Fink, 33c), and of normal dogs (Dobriner, 23d).

If the 4 methyl groups in coproporphyrin type I are carboxylated a compound is formed which is called uroporphyrin type I. This is

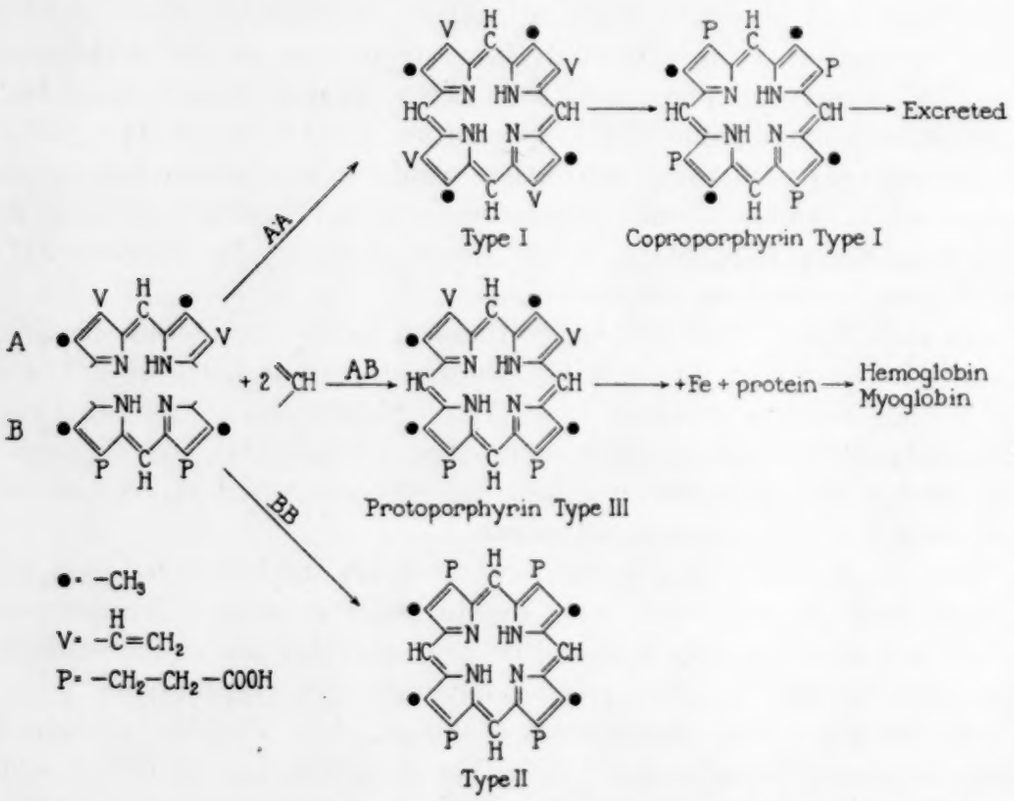

Fig. 4. A scheme representing the simultaneous synthesis of the type I and type III porphyrins.

present in large amounts in the urine of patients with congenital porphyria, and was isolated with coproporphyrin I by Fischer (34a, b, c).

It is important to realize that a transformation of one type of porphyrin into another by a change in the positions of the substituted groups is not possible chemically. It could be done only by degradation of the porphyrin complex to simple pyrrols, followed by resynthesis. There is no evidence so far that such a process occurs in human beings

or in animals under any circumstances. Hence Fischer suggested that the two types are synthesized independently (34).

In a study of the coexistence of two basically different porphyrin types, the so-called "dualism," Dobriner isolated and identified the types of porphyrins excreted in the urine and feces in over 100 cases of disease as well as in normal human beings and animals (23).

Early in the course of the studies by qualitative methods it was observed that there seemed to be a relation between increased hematopoietic activity and increased coproporphyrin I excretion. Quantitative studies were then begun. Finally a working hypothesis was formulated (23f, h, l, m) to explain the dualism. This explanation of the concurrent construction of types I and III porphyrins is represented schematically in figure 4.

As stated previously, porphyrins are synthesized by the combination of substituted pyrrols to form suitably constructed pyrromethenes, which are then condensed to porphyrins.

If one considers the two pyrromethenes A and B formed from substituted pyrrols, as joined together by two methene groups to form porphyrins, there are three possible combinations: 1, two molecules of A may unite, or 2, two molecules of B, or 3, one molecule each of A and B. As can be seen from the figure, the following compounds would form: (1) 1,3,5,7,tetramethyl 2,4,6,8,tetravinyl porphyrin, (2) 1,3,5,8,tetramethyl 2-4 divinyl-6-7 dipropionyl porphyrin, and (3) 1,4,5,8,tetramethyl 2,3,6,7,tetrapropionyl porphyrin. If the order of substitution is compared with the etioporphyrins in figure 3, it will be seen that A.B. is a type III, A.A. is a type I, and B.B. is a type II, porphyrin.

The individual compounds will be formed in relatively different amounts according to the conditions under which the synthesis operates, and to the relative amounts of A and B which take part.

The type II porphyrin which should be formed according to the outline presented has not yet been demonstrated in nature. This is due perhaps to its failure to be formed at all in the biological synthesis, or to the great difficulty of obtaining sufficiently large amounts of the compound to allow separation and identification.

Fischer tried to direct the *in vitro* syntheses in such a way as to obtain only one type of porphyrin, but in many instances mixtures of different kinds and types resulted ("mixed synthesis") (34).

In vivo, under normal conditions, the porphyrin synthesis is directed to the formation of relatively large amounts of type III porphyrins, and

small amounts of type I. The rate of production of type I seems to be in a definite ratio to the rate of formation of the type III compounds which later combine with iron and specific proteins to form respiratory pigments.

Under certain conditions there may occur an orderly increased or decreased formation of type III porphyrins associated with a simultaneous increase or decrease of type I production. Under other conditions this constant ratio may be disturbed with a resultant disorderly increased formation of type I compounds and often with the production of abnormal porphyrins as is the case in congenital porphyria.

This theory was first advanced in a preliminary communication concerning congenital porphyria in 1936 (23f), and experiments in human beings and animals were in progress at that time to prove its validity (23, b–o). Rimington independently advanced a similar hypothesis as an "enzymatic theory of hematopoiesis" (118a, c).

The isolation of porphyrins: their chemical and physical properties. Detailed descriptions of the chemical and physical properties of the porphyrins, with information regarding methods of analysis and synthesis, are found in the monograph of Fischer-Orth (34).

The porphyrins are of reddish color in acid solution, and in organic solvents show intense absorption bands in the visible region of the spectrum (Schumm, 130a; Fischer and Treibs, 35a; Kirstahler, 76). The position and number of the bands depend upon the pH and temperature as well as on the solvents, a subject investigated at low temperatures by Conant and Kamerling (19). The different kinds of porphyrins have absorption bands in different positions but the bands of isomeric compounds are in the same positions and therefore cannot be used for the identification of types.

In ultraviolet region of the spectrum the porphyrins show an intense absorption band in the region of 3900 to 4100 Å. They also show a very intense red fluorescence, as well as specific bands by fluorescence spectroscopy (Dhéré, 22; Borst and Königsdörfer, 12). In acid solution, the fluorescence may be detected in extreme dilutions and in this way minute amounts of porphyrins in organs and tissues may be detected. The relation of absorption to the constitution of the porphyrins is discussed in detail in the publications of Stern and Wendelein, Stern and Molvig, and Pruckner and Stern (literature, 34, p. 579). The relation of the intensity of fluorescence to the pH has been used for the identification of isomeric porphyrins by Fink and Hoerburger (33e, f).

Protoporphyrin, mesoporphyrin, deuteroporphyrin, and the copro-

porphyrins are all soluble in a mixture of acetic acid and ether, but uroporphyrin types I and III are not. Deuteroporphyrin and protoporphyrin are chloroform-soluble under certain conditions. Uroporphyrin type III is soluble in ethyl acetate, but uroporphyrin type I is not dissolved by this compound or by acetic acid. All porphyrins are soluble in strong mineral acids. The protoporphyrin, deuteroporphyrin, and hematoporphyrin form insoluble sodium salts and can thus be separated from the coproporphyrins. The ether-soluble porphyrins can be separated from ether by extraction with different concentrations o hydrochloric acid. The concentrations are called the specific HCl

TABLE 1

Physical constants of important porphyrins (34)

	HCl NO*		MELTING POINTS OF METHYL ESTER	SOLUBILITY OF FREE PORPHYRIN IN NaOH
	Free porphyrin	Methyl ester		
			°C.	
Protoporphyrin IX	2.0	5.5	228	Insoluble
Mesoporphyrin IX	0.5	2.5	216	Insoluble
Deuteroporphyrin IX	0.4	2.0	223	Insoluble
Hematoporphyrin IX	0.1		212	Insoluble
Coproporphyrin I	0.08	1.5	252	Soluble
Coproporphyrin III	0.09	1.5	135† 170	Soluble
Uroporphyrin I		7 ca	302	Soluble
Uroporphyrin III		7 ca	256	Soluble

* The concentration of hydrochloric acid which will remove two-thirds of the porphyrin from an ether solution of the porphyrin when equal volumes are shaken together.

† Double melting point.

numbers (Willstätter). Some of the physical constants are given in table 1.

The coproporphyrins, uroporphyrins, deuteroporphyrins and mesoporphyrins, are identified as their methyl esters. Protoporphyrin undergoes a change of the labile vinyl groups during the processes of isolation and separation (Fischer, Treibs and Zeile, 35i), and it is reduced to mesoporphyrin for identification by the method of Fischer and Pützer (35b).

A detailed description of the methods used up to 1927 is presented by Fischer (34e), and since then Fischer and Duesberg (35m), Watson

(156f), Waldenström (155a, b), Dobriner (23b, k), and Rimington (118h) have reported modifications and improvements based on Fischer's procedures. The physical constants for the separation of the natural porphyrins have been described recently in the papers by Zeile and Rau (161a), and by Keyes and Brugsch (75, 15f). In table 2 a scheme for the separation of the fecal porphyrins is given which, with slight modifications, can be applied to those in urine (23k). For the isolation of uroporphyrin type I the method of Fischer and Duesberg (35m) should be used, and for uroporphyrin type III that of Waldenström (155a, b).

It has become increasingly important to separate different isomers. The methyl ester of coproporphyrin type III can be crystallized only with difficulty. Up to 1936 the usual method for identification of the important coproporphyrin was to recrystallize the methyl ester several times and thus raise the melting point. It seems certain that in many instances the presence of small amounts of isomeric compounds was overlooked. Dobriner (23b, c) introduced a simple method for the separation of the methyl esters of coproporphyrin types I and III. Rimington (118h) employs absolute ethyl ether instead of methyl alcohol.

For all measurements a quantitative separation of the porphyrins is essential. With the exception of a few conditions in which ether insoluble uroporphyrins are present, the acetic acid-ether method should be used. If proto-, deutero- or mesoporphyrins are to be determined, the patients should be on a hemoglobin- and myoglobin-free diet. The determinations of the content in either urine or feces should be made if possible on 3-day collections over a 9-day period. Many investigators in the past have only studied urine, but it seems to be essential to examine the feces also. Twenty-four hour collections of urine should be used because of the different concentrations of porphyrins excreted at different times. After isolation, purification, and separation of the different kinds of porphyrins, quantitative determinations may be made by spectroscopic (130a, 23k), spectrocolorimetric (17a, 156f), spectrophotometric (130a, g), photoelectric (23l), and fluorometric (152c, 15b, 32b) methods. The results of the determinations with the different procedures agree reasonably well, although fluorometric methods seem to give slightly lower values. Fluorometric procedures are essential for the determination of the protoporphyrin in blood.

The porphyrins in nature: It is not possible in this review to mention all the publications concerning this subject. The iron porphyrin com-

TABLE 2

A scheme for the separation, purification and identification of the porphyrins of feces

I. Extraction of the total crude porphyrins with ether. Purification of total porphyrins		
II A. Saponification of the natural porphyrin esters with 20% NaOH. Separation of soluble and insoluble sodium salts		
III a. Soluble sodium salts, *coproporphyrins*	II b. Insoluble sodium salts, *deuteroporphyrin* and *protoporphyrin*	
	II B. Separation of deutero- and protoporphyrins with 0.6% HCl from ether	
	0.6% HCl fraction, deuteroporphyrin (III B)	Ether fraction, protoporphyrin (III C)
Purification		
III A. *Coproporphyrins*	III B. *Deuteroporphyrin*	III C. *Protoporphyrin*
Extracted from ether with 0.2% HCl	Extracted from ether with 0.6% HCl	Remains in ether after extraction with 1.0% HCl
Purification with chloroform and petroleum ether	HCl concentration reduced to 0.2% and extracted with chloroform	Extracted with 5% HCl and dissolved in chloroform
Coproporphyrins in HCl	Deuteroporphyrin in chloroform	Protoporphyrin in chloroform
Identification		
IV A. As coproporphyrin methyl esters	IV B. As deuteroporphyrin methyl esters	IV C. As protoporphyrin or mesoporphyrin methyl esters
V. Separation of coproporphyrin I and III methyl esters with methyl alcohol		
Methyl alcohol		
Soluble	Insoluble	
Coproporphyrin methyl ester III	Coproporphyrin methyl ester I	

(156f), Waldenström (155a, b), Dobriner (23b, k), and Rimington (118h) have reported modifications and improvements based on Fischer's procedures. The physical constants for the separation of the natural porphyrins have been described recently in the papers by Zeile and Rau (161a), and by Keyes and Brugsch (75, 15f). In table 2 a scheme for the separation of the fecal porphyrins is given which, with slight modifications, can be applied to those in urine (23k). For the isolation of uroporphyrin type I the method of Fischer and Duesberg (35m) should be used, and for uroporphyrin type III that of Waldenström (155a, b).

It has become increasingly important to separate different isomers. The methyl ester of coproporphyrin type III can be crystallized only with difficulty. Up to 1936 the usual method for identification of the important coproporphyrin was to recrystallize the methyl ester several times and thus raise the melting point. It seems certain that in many instances the presence of small amounts of isomeric compounds was overlooked. Dobriner (23b, c) introduced a simple method for the separation of the methyl esters of coproporphyrin types I and III. Rimington (118h) employs absolute ethyl ether instead of methyl alcohol.

For all measurements a quantitative separation of the porphyrins is essential. With the exception of a few conditions in which ether insoluble uroporphyrins are present, the acetic acid-ether method should be used. If proto-, deutero- or mesoporphyrins are to be determined, the patients should be on a hemoglobin- and myoglobin-free diet. The determinations of the content in either urine or feces should be made if possible on 3-day collections over a 9-day period. Many investigators in the past have only studied urine, but it seems to be essential to examine the feces also. Twenty-four hour collections of urine should be used because of the different concentrations of porphyrins excreted at different times. After isolation, purification, and separation of the different kinds of porphyrins, quantitative determinations may be made by spectroscopic (130a, 23k), spectrocolorimetric (17a, 156f), spectrophotometric (130a, g), photoelectric (23l), and fluorometric (152c, 15b, 32b) methods. The results of the determinations with the different procedures agree reasonably well, although fluorometric methods seem to give slightly lower values. Fluorometric procedures are essential for the determination of the protoporphyrin in blood.

The porphyrins in nature: It is not possible in this review to mention all the publications concerning this subject. The iron porphyrin com-

TABLE 2

A scheme for the separation, purification and identification of the porphyrins of feces

I. Extraction of the total crude porphyrins with ether. Purification of total porphyrins		
II A. Saponification of the natural porphyrin esters with 20% NaOH. Separation of soluble and insoluble sodium salts		
III a. Soluble sodium salts, *coproporphyrins*	II b. Insoluble sodium salts, *deuteroporphyrin* and *protoporphyrin*	
	II B. Separation of deutero- and protoporphyrins with 0.6% HCl from ether	
	0.6% HCl fraction, deuteroporphyrin (III B)	Ether fraction, protoporphyrin (III C)
	Purification	
III A. *Coproporphyrins*	III B. *Deuteroporphyrin*	III C. *Protoporphyrin*
Extracted from ether with 0.2% HCl	Extracted from ether with 0.6% HCl	Remains in ether after extraction with 1.0% HCl
Purification with chloroform and petroleum ether	HCl concentration reduced to 0.2% and extracted with chloroform	Extracted with 5% HCl and dissolved in chloroform
Coproporphyrins in HCl	Deuteroporphyrin in chloroform	Protoporphyrin in chloroform
	Identification	
IV A. As coproporphyrin methyl esters	IV B. As deuteroporphyrin methyl esters	IV C. As protoporphyrin or mesoporphyrin methyl esters
V. Separation of coproporphyrin I and III methyl esters with methyl alcohol		
Methyl alcohol		
Soluble	Insoluble	
Coproporphyrin methyl ester III	Coproporphyrin methyl ester I	

pounds and iron-protein-porphyrin compounds (respiratory pigments and enzymes) cannot be discussed, nor can the bile pigments. Excellent reviews have been written by Anson and Mirsky (2), McMaster (103), Fischer (34), and Watson (156).

Protoporphyrin number 9 (type III) is identical with Kämmerer's prophyrin, with Fischer's oöporphyrin, and with Schumm's hematerinic acid. It was the one obtained from the blood of Petry, who excreted a large amount of type I porphyrins. Recently Fischer (34d) announced the formation of mesoporphyrin numbers 2 and 9 from the protoporphyrin of natural heme. These are types I and III porphyrins respectively.

Protoporphyrin was produced by the bacterial fermentation of blood (Kämmerer, 72a), by the sterile autolysis of myoglobin (Hoagland, 63) and by treating reduced hemoglobin with concentrated hydrochloric acid. By treating hemochromogen with weak acids, the iron-protein part can be split off from the respiratory pigments with the production of free protoporphyrin (Schumm, 130a). If hemoglobin or myoglobin is digested in the intestinal tract, 89.5 per cent protohemin, 2.8 per cent protoporphyrin, 6.6 per cent deuterohemin and 0.9 per cent deuteroporphyrin, are formed (Haurowitz, 56a, b). The publications by Snapper (136), Schumm (130a) and Boas (11a, b) regarding the metabolism of hemoglobin in the intestine should be consulted. In the feces of normal human beings on a meat-free diet only traces of protoporphyrin are found. Among animals, rats excrete large amounts of protoporphyrin (118g) and it was isolated by Rimington from normal sheep liver (118b).

Fischer and Kögl (34j) isolated and identified protoporphyrin type III from the colored egg shells of different species of birds. Hijmans v. d. Bergh and Grotepass observed during the incubation of hen eggs the production of free protoporphyrin (61d). Its presence in cereals and other plant material (35c), as well as in fresh yeast, is reported by Fischer and co-workers (34n, 102) and by Schumm (130a).

Deuteroporphyrin number 9 (type III) is identical with Schumm's copratoporphyrin, and was isolated and identified from feces by Watson (156a). It is a disintegration product of the protoporphyrin of hemoglobin. Watson (156f) found related compounds which he called pseudo-deuteroporphyrins A and B. Fischer and Duesberg (35m) stated that they had isolated substances like deuteroporphyrin from the feces of rabbits, but never conclusively identified them as deuteroporphyrin, since they showed much lower melting points than deuteroporphyrin number 9.

Mesoporphyrin number 9 was isolated and identified recently by Zeile and Rau (161a), and Grotepass and Defalque (48e) from the feces of human beings.

The presence of coproporphyrin in biological material has often been reported. Identification of types was rarely possible. Fischer and co-workers reported the presence of coproporphyrin in seeds and plant material (34, 35c), and Kämmerer and Gürsching (72c) in foodstuff. In bacilli and fungi coproporphyrin is present in small amounts, as shown by Fischer and Fink (34n), Coulter and Stone (20), and Carrié and Mallinckrodt (17a, 17c, 94). Jakob recently isolated coproporphyrin type III from bacterial cultures (70b).

The presence of coproporphyrin I in urine and feces of normal and diseased individuals is discussed later in detail. Dobriner (23d, g) isolated it from the excreta of normal dogs. Fischer and co-workers (34, 34n), Fink (33a) and Mayer (102), reported the presence of coproporphyrin type I in yeast, and heme of type III construction was also identified. If yeast is poisoned with copper large amounts of coproporphyrin I are produced. Schønheyder (128) isolated coproporphyrin type I from eggs and followed the rate of its production during incubation. Coproporphyrin type III has been found in the urine of normal rabbits (Dobriner and Rhoads, 23j), and its presence in nature is reviewed by Rimington (118d).

Uroporphyrin type I is found in the urine of human beings and animals with congenital porphyria, but is not present in normal urine (Grotepass, 48d). Fischer (34), with Haarer (35l) and with Hofmann (35r), found it in mussel shells (pteria), and Waldenström (155a) confirmed this, but in two instances he found uroporphyrin which gave much lower melting points and different fluorescence curves. This suggests that the compound is intermediate between uroporphyrin types I and III. From the colored feathers of the bird Turacus, Fischer and Hilger (34i) isolated uroporphyrin type I. Turner (148a) found uroporphyrin type I in the skeleton of the fox squirrel.

Uroporphyrin type III was isolated by Waldenström (155a, e) from the urine of a large number of cases of acute porphyria. Völcker (154a, b), and independently Rimington (118e), isolated uroporphyrin III from many species of tropical bird feathers.

Bingold (8) described in the urine a pigment called pentdyopent, which can be artificially produced by the action of hydrogen peroxide on hematin or bile pigments, and Fischer and Müller (35s) identified it as a pyrromethene.

The physiology of the porphyrins. The photosensitizing action of

the porphyrins was discovered by Hausmann in 1910, and since then many experiments have been published. The reviews by Hausmann and Haxthausen (57a), Ellinger (28), and Blum (10a, b, c), should be consulted. There seems to be a difference in the action of the same porphyrin in different species (34, 35e). Hematoporphyrin has received special attention. The action of the naturally occurring porphyrins, protoporphyrin type III, coproporphyrin, and uroporphyrin types I and III, requires further study. Coproporphyrin type I sensitizes less than uroporphyrin type I, and the effect of uroporphyrin type III has not been studied. Coproporphyrin type III produces less light sensitivity than does type I (9b).

Hausmann and Kuen (57b) reported the effect of different kinds of porphyrins on the hemolysis of erythrocytes. "Light shock" after the injection of porphyrin was studied by Rask and Howell (116) and Smetana (135a, b, c), who also investigated the photo-oxidation of hematoporphyrin in the animal organism. In a famous experiment Meyer-Betz (106) injected himself with 200 mgm. of hematoporphyrin, and developed after exposure to light a severe edema of the skin of the exposed regions. The lesions are different from those of congenital porphyria, and light sensitivity continued for a considerable period.

Hinsberg and Merten (62c) observed a 50 to 100 per cent increase in the rate of protein metabolism after the injection of 4 mgm. of hematoporphyrin daily. The C:N ratio increased from 1.3 to 4.3, and the O:N ratio from 5.5 to 8.7. An increased excretion of chlorides was also present. Hinsberg and Ammon (62b) studied the influence of porphyrins on the auto-oxidation of fatty acids.

Gaffron (40), Boyd (13), Smetana (135a, b, c), and Holden (66a, b), observed that porphyrins combine with proteins (fibrinogen, albumin, and globin). Light hydrolyzes the proteins in the presence of porphyrin, and oxygen is necessary for this reaction. Gildemeister (44) employed cataphoresis in the study of the combination of serum proteins with porphyrins. Hematoporphyrin number 9, as well as coproporphyrin types I and III, combine with the albumin fraction but uroporphyrin types I and III do not do so. These findings are of great interest since Shibuya (133), as well as Rask and Howell (116), found that serum protects against photosensitization (57a).

The destruction of the porphyrins in tissues has been the subject of several studies. Sumner (142) observed that hematoporphyrin is destroyed *in vitro* by liver. Perutz (114) mixed liver with the urine of rabbits poisoned with sulfonal. This urine was high in its content of

porphyrin. Porphyrin could not be recovered from the mixture. Similar experiments were performed by Schreus and Carrié (129b) by adding liver tissue to the concentrated urine of patients with acute porphyria. The destructive effect could be abolished by heating the tissue to 56°C. Hijmans v. d. Bergh and co-workers (61b) perfused liver with solutions of protoporphyrin in defibrinated blood. The bile was shown to contain coproporphyrin, but none was formed when protoporphyrin was absent from the perfusion fluid. Coproporphyrin so produced can be only of type III configuration.

Little is known concerning the metabolism and quantitative excretion of the naturally occurring porphyrins, when administered parenterally or orally. This is due to the fact that accurate quantitative methods for porphyrin separation and determination in feces have been made available only recently. Fischer showed that, in rabbits, injected coproporphyrin type I is excreted in both the urine and the feces, but that uroporphyrin type I is only excreted in the urine (34). Vigliani (153f) observed that from patients with biliary fistulae only about 50 per cent of coproporphyrin type I and 20 per cent of type III injected intravenously could be recovered. Dobriner (23d), in similar experiments in dogs, recovered about 70 per cent of coproporphyrin type I in 24 hours. Fischer (34k), Schumm (130d), and Watson (156j), recovered an increased amount of coproporphyrin in the urine after administering protoporphyrin. The fate of hematoporphyrin has been investigated in more detail, since that compound has been used in the treatment of manic-depressive psychoses (68, 39). Neubauer (112) after injection recovered this compound from the bile. Similar experiments in animals are reported by Hutschenreuter (69), Jakob (70a), and Smetana (135c), and in human beings by Häcker (51), Jakob (70a), and Schumm (130g). All the investigators agree that only traces of injected hematoporphyrin are found in the urine. Grotepass and Hulst (48b) perfused kidneys and observed that uroporphyrin is excreted rapidly, coproporphyrin less so, and protoporphyrin not at all. Schumm (130a), Derrien and Cristol (21), and Kapp (73a), reported the presence of complex zinc and copper salts of porphyrin in the urine.

Fischer (34c) suggested that the toxic action of the porphyrins depends upon the number of carboxyl groups. In white mice the order of toxicity, as judged by the severity of the "light shock" produced, is: hematoporphyrin number 9, uroporphyrin type I, deuteroporphyrin number 9, coproporphyrin type I, and protoporphyrin number 9.

Coproporphyrin type III seems to be slightly toxic and uroporphyrin type III non-toxic.

The action on the intestinal tract of hematoporphyrin number 9, coproporphyrin type I, and uroporphyrin type I, was studied by Reitlinger and Klee (117), Supniewski (143), and Vannotti (152b). These compounds increased the tonus and the effect was not abolished by atropine. The action of porphyrins on the circulation was studied by Supniewski (143), Rask and Howell (116), Smetana (135a), and Vannotti (152b).

The impregnation with uroporphyrin type I of the bones and teeth in congenital porphyria has suggested experiments in animals. It is important to note that from cases of acute porphyria excreting large amounts of uroporphyrin type III, neither Waldenström (155a) nor Mertens (105c) could isolate that substance from the bones. Fraenkel (37), Hammer (54), Borst and Königsdörfer (12), Fikentscher (32d), and others found that in the bones of embryonic, as well as newborn, human beings and animals small amounts of porphyrin are present, chiefly in the zones of calcification. In experiments with growing animals these authors, as well as Fikentscher et al. (32g), followed the fate in the bones of injected porphyrin. Uroporphyrin type I is easily and quickly deposited, as is hematoporphyrin number 9 if injected in large amounts. Coproporphyrin type I, mesoporphyrin number 9, and protoporphyrin number 9, seem to have little or no tendency to deposit. Bingel (9b) proved that uroporphyrin type III is deposited in bones and teeth. Emminger and Battestini (30a) studied the bones of animals with lead poisoning, and found that they contained none of the coproporphyrin type III excreted. The rate of elimination of different porphyrins seems to vary. The bones of the fetus are not impregnated with the porphyrins injected into the mother (12, 37, 54).

Van Leersum (151) described a therapeutic effect of injecting hematoporphyrin into rats with rickets, but Emminger and Büchele (30b), and Marique (96), could not confirm this observation. The natural porphyrins have not been studied in this regard. Strauch (140) discusses rickets in children and its relation to porphyrins.

Hinsberg and Rodenwald (62d) reported experiments concerning the action of hematoporphyrin and protoporphyrin on the hypophysis of frogs, rats, rabbits, and dogs. An increased excretion of melanophore-dispersing hormone was observed. In the serum of injected animals a substance was found which inactivated the hormone, and the authors

believe that the inactivating agent is the one found in the serum of patients with cancer.

Hinsberg (62a) reported recently that after the injection of hematoporphyrin and protoporphyrin in infantile mice, premature follicle formation in the ovaries takes place.

Porphyrins in normal human beings. Coproporphyrin is always present in small amounts in the urine, and was identified by Hoerburger (64a), Fink and Hoerburger (33g), and by Fink (33c), as coproporphyrin I, a finding confirmed by Watson (156e), by Dobriner (23e), and by Zeile and Rau (161a). Watson's and Fink's results suggest the presence of small amounts of coproporphyrin III. Grotepass (48d) isolated from 10,000 liters of pooled normal urine a mixture of equal parts of coproporphyrin types I and III. The writers believe that whereas traces of type III may be present, they have not succeeded so far in identifying the compound from normal urine. Pooled urine may have included specimens from individuals suffering from disorders marked by the excretion of the type III compound. Grotepass did not find any protoporphyrin or uroporphyrin in the urine of normal individuals.

In the feces the coproporphyrin found by Fischer and Schneller (34f) by spectroscopic methods was isolated and identified as type I coproporphyrin by Watson (156f), and by Dobriner (23b). Coproporphyrin III so far has not been isolated from the feces of normal individuals, but some of the melting points reported suggest that possibly traces of this substance may have been present. Watson (156a) isolated deuteroporphyrin number 9 from the feces of a patient taking meat.

Watson (156f) isolated and identified the coproporphyrin present in bile as type I. Vigliani (153f) did not find any protoporphyrin in the bile.

In table 3 the amounts of porphyrin contained in normal urine are listed as reported by different investigators. The results obtained by the different methods correspond closely. Determinations of coproporphyrin in the feces have been reported by only a few workers, and it is to be deplored that in the extensive early work of Brugsch (15b, c, d) the porphyrins isolated were not differentiated at all. Vigliani and co-workers (153e, f, g) made rough estimates of the ratio between excreted coproporphyrin and protoporphyrin. Dobriner and coworkers (23b, l) measured the excretion of coproporphyrin in the urine and feces of normals for 9-day periods. The results are given in table 4. The total daily urinary and fecal excretion by adults varied between

306 and 376 micrograms, with an average of 350 daily. Brugsch (15a) states that normals excrete daily from 150 to 300 micrograms. Children appear to excrete less coproporphyrin than do adults (Dobriner et al., 23 l).

TABLE 3

The average daily excretion of coproporphyrin in the urine of normal individuals as determined by different investigators by different methods

AUTHORS AND REFERENCES	URINARY COPROPORPHYRIN LEVEL FOR 24 HOURS	METHODS APPLIED
	mcg.	
Franke and Fikentscher (38b)	10–30 (60)	Fl.
Hijmans v.d. Bergh et al. (61b)	10–100	Fl.
Brugsch (15a, b)	4–50 (80)	Fl.
Tropp and Siegler (147a)	18–110	Fl.
Vigliani et al. (153e)	6–46	Fl.
Vannotti (152a, c)	10–80	Fl.
Schreus and Carrié (17a)	0–60 (80)	Sp.col.
Lageder (79a)	0–100	Sp.col.
Thiel (145a, b)	0–100	Col.
Dobriner et al. (23b, k, l)	41–120 (143)	Ph.el.col.

Fl. = fluorimetric; Sp.col. = spectro-colorimetric; Ph.el.col. = photoelectric colorimetric; Col. = colorimetric.

TABLE 4

Quantitative coproporphyrin I excretion in micrograms in average daily values for 9-day period

CASE NUMBER	AGE	SEX	R.B.C.	HGB. PER 100 CC.	COPROPORPHYRIN		
					Urinary	Fecal	Total
			millions	*grams*	γ	γ	γ
1	12	F	4.5	15	35	191	226
4	21	M	4.6	13	87	231	318
2	25	M	4.5	15	123	205	328
5	33	M	4.59	14.4	102	274	376
3	35	M	5.08	15.9	121	241	362
6	72	M	4.3	14.8	64	242	306

The presence of protoporphyrin in blood was determined quantitatively by Hijmans v. d. Bergh (61b), who found that 8 to 12 micrograms are present in 100 cc. of normal blood. Findings in normals and in disease are reported by Lageder (79b), Vigliani and Angeleri (153b),

Vannotti (152b), and recently by Schumm (130g), who found average values of 13 to 15 micrograms per cent. Watson and Clarke (156h) demonstrated protoporphyrin in the reticulocytes, and Grotepass (48c) identified that in erythrocytes as protoporphyrin number 9 (type III). In the serum under normal conditions coproporphyrin does not seem to be present (Schumm, 130g).

The presence of protoporphyrin, and possibly of small amounts of other porphyrins, in the bone marrow of normal human beings and in pathological conditions is described by Borst and Königsdörfer (12). The megaloblasts and erythroblasts seem to contain a relatively large amount of porphyrin.

Günther (50a) detected traces of coproporphyrin in meconium and it was identified by Waldenström (155a) as type I. Fikentscher (32c) found coproporphyrin in the amniotic fluid. Since injected porphyrins do not pass through the placenta (54, 37) it must be assumed that the embryo synthesizes coproporphyrin type I. Fikentscher (32d) investigated the content of coproporphyrin in the serum of embryos and newborn infants. It was present in the last month of pregnancy, and at birth. Herold (60a) studied the excretion of coproporphyrin by newborn infants with icterus neonatorum.

No increase of the rate of excretion of coproporphyrin during normal pregnancy was found by Carrié and Herold (17b), by Herold (60b), or by Fikentscher (32e). In toxemia of pregnancy and in hyperemesis gravidarum an increase was observed.

Porphyrias. These conditions make up a group of congenital diseases marked by a disturbed metabolism of pyrrols and by the excretion of large amounts of porphyrins. The etiology is unknown. Günther (50a, b) classified the different sub-groups according to their clinical manifestations; Micheli and Dominici (107), and Carrié (17a), on a different basis. Waldenström (155a) employed Günther's terminology, and it will be followed in this paper. Three types of disorder have been described: 1. Congenital porphyria (Günther). 2. Acute porphyria (Günther-Waldenström). 3. Chronic porphyria.

Great sensitivity to light and the excretion of large amounts of type I porphyrins are features of congenital porphyria. Acute porphyria is also a congenital disease, but the patients manifest no light sensitivity, and uroporphyrin type III is excreted. Cases of a type intermediate between the first two have been described by Günther as chronic porphyria, in which either type I or type III porphyrin is excreted. Porphyria must be distinguished from porphyrinuria, a

term which simply means the excretion of abnormally great amounts of porphyrins in the urine.

Congenital porphyria. Congenital porphyria was reviewed by Günther in 1922 and 1925 (50a, b), by Mason et al. (98), Vannotti (152a, b), Watson (156i), and by Turner and Obermayer (148c). The last authors discussed 86 cases of which 9 were considered to be doubtful. Dobriner and co-workers (23o, 113), reported chemical studies of 3 cases. A detailed report of the post mortem findings in the famous patient Petry is to be found in the monograph by Borst and Königsdörfer (12).

Congenital porphyria is a rare inborn error of metabolism (Garrod, 41a) possibly inherited as a Mendelian recessive (Günther, 50a, b; Mackey and Garrod, 93, 41b). The classical manifestations are: 1, the excretion of large amounts of porphyrin; 2, discoloration of the teeth and bones by impregnation with uroporphyrin I; 3, sensitivity of the skin to light in the spring and summer, a symptom which often appears first in childhood. Blistering of the exposed areas of the face and extremities is observed, and the lesions heal with scar formation, followed in many cases by deformity of the affected tissue. Limited space precludes a discussion of the clinical manifestations, but certain symptoms of endocrine disturbance deserve mention. In many cases hirsutism was present—for example, in Hegler's and Fraenkel's "bearded lady" (59). Morphological changes of the endocrine glands of Petry were described by Borst and Königsdörfer (12).

The disease ochronosis in animals was discovered by Schmey (126) in 1913 to be congenital porphyria. A review of the literature concerning this disorder is given by Fourie (36a), and by Fink (33b). Uroporphyrin type I was isolated from the bones of swine with porphyria by Fink and Hoerburger (33d). Fourie (36a) with Rimington (36b) reported congenital porphyria in living cattle.

The etiology of congenital porphyria is unknown. Dobriner and co-workers (23f, o), and Rimington (118a, c), independently advanced similar explanations of the disordered pigment metabolism, as follows: In congenital porphyria the normal ratio between the formation of type I and type III porphyrins is disturbed to give a disproportionate or disorderly type of synthesis in favor of type I. The disturbance is quite unlike any other disorder of pyrrol metabolism so far observed in human beings or in animals.

The relation of the porphyrins to light sensitivity is discussed elsewhere, and the fact is stressed that the different types and kinds of

porphyrins vary considerably in their photosensitizing action. In congenital porphyria Borst and Königsdörfer (12) and Carrié (17a) obtained evidence by fluorometric methods that porphyrins are deposited in the skin. Experiments with animals indicate that uroporphyrin type I, when injected in pure solution, sensitizes to light. Many investigators have attempted to produce artificially the skin lesions of congenital porphyria by exposing the patients to light from artificial sources. No successful experiments are reported. The literature is reviewed by Perutz (114), Günther (50a, b), Hausmann and Haxthausen (57a), Carrié (17a), Gottron and Ellinger (46a, b), and by Blum (10a, b, c). It has been suggested that in congenital porphyria the excretion of a brown pigment of nonporphyrin nature caused the disorder, since if this pigment is injected into animals simultaneously with type I porphyrins, no light sensitivity occurs (34h). Many studies have been made of the effect of hematoporphyrin number 9 on human beings and animals, but no condition like congenital porphyria has resulted. No experiments have employed uroporphyrin type I combined with coproporphyrin type I, the compounds which are excreted in congenital porphyria.

The early investigators often did not distinguish between congenital porphyria and hydroa aestivale or vacciniforme. In 1937 Mathews (100) collected 57 cases of hydroa aestivale; of these patients the urine of only 32 was examined for porphyrin. It was present in 22, but no clear evidence proves that these cases were true congenital porphyria. The authors recently studied a severe and typical case of hydroa vacciniforme. The urine and feces contained a normal amount of coproporphyrin type I (23e), and no abnormal kinds of porphyrins could be detected, a finding which is in agreement with Günther's (50a) early report. It should be emphasized that hydroa aestivale and vacciniforme are only symptom complexes and that congenital porphyria with similar skin lesions is a well-defined disorder of pyrrol metabolism.

The urine excreted in congenital porphyria is of a Burgundy red color, with a brown tinge, the latter due to a pigment isolated by Fischer et al. (34h). It contains large amounts of coproporphyrin type I and uroporphyrin type I, as proved first in 1915 by Fischer (34c). In many cases the urine darkens considerably after exposure to light, a phenomenon which is due to the photo-oxidation of leucoporphyrins. The facts concerning the pigments excreted in the urine of 9 patients are summarized in table 5. In 1936 Fischer and Libowitzky (35p) isolated from a large amount of uroporphyrin type I a small amount of

type III compound, and in 1939 Mertens (105d) found in the coproporphyrin fraction of the same patient much type I and a small amount of type III. The melting points reported by other investigators indicate the presence in their material of small amounts of isomers.

TABLE 5

The melting points of the methyl esters of the porphyrins excreted by the reported patients with congenital porphyria

	CHEMICAL WORK UP	REFERENCE	CLINICAL WORK UP	REFERENCE	MELTING POINT OF PORPHYRINMETHYLESTERS, °C.					
					Urine				Feces	
					Uroporphyrin		Coproporphyrin		Coproporphyrin	
					Type I	Type III	Type I	Type III	Type I	Type III
1	Fischer	34a, b, c	Günther	50a, b	293		250		249	
			Weiss	158						
	Fischer-Hofmann	352	Borst-Königsdörfer	12	302	261				
	Mertens	105d	"Case Petry"						252	206*
2	Fischer-Zerweck	34	"Case Molzberger"	34	275				247/51	
3	Allot	93	Mackey-Garrod	93	283				248	
4	Allot	3	Ashby	3	286				245	
5	Jost	71	Schmidt-La Baume	71	272					
6	Waldenström	155a	"Case Schlosser"	155a	289		245		245	
7	Dobriner et al.	23o	Peachey et al.	113	286		250		249	
8	Dobriner et al.	23o	Guild	Not pub.	285		251		250	
9	Dobriner et al.	23o	Hardgrave	Not pub.	279		249		241	
10‡	Rimington et al.	118a, i	Fourie	36a, b	293	260	233/35	138† 172	250	
Melting points of the analytic methylesters (34)					302	261	252	135† 170	252	206*

* M.P. of Cu. methylester.
† Double melting point.
‡ In cattle.

In the feces of two patients Dobriner et al. (23o) found only traces of protoporphyrin and deuteroporphyrin; from one a conjugated coproporphyrin I was isolated.

The presence of coproporphyrin in the serum was observed by several investigators (Fischer, 34; Schumm, 130a). The hemoglobin of Petry contained normal protoporphyrin number 9, a type III compound. Fischer (34c) and Schumm (130b) reported some quantitative studies of the amount of coproporphyrin and uroporphyrin excreted by

Petry, and similar studies of two children are recorded by Dobriner and co-workers (23o).

Fischer and co-workers (34m) isolated and identified uroporphyrin type I from the bones and kidneys of Petry, as well as coproporphyrin type I from the bile, which contained no uroporphyrin. Spectroscopically, uroporphyrin and coproporphyrin were present in the bone marrow, liver, and kidney. In the blood plasma and in the intestine, coproporphyrin but no uroporphyrin, was seen spectroscopically.

Duesberg (25b) in 1931 suggested the treatment of congenital porphyria with liver extract. Schreus and Carrié (77a), as well as Vannotti (152b), published evidence that the administration of liver extract decreased the rate of porphyrin excretion. Dobriner and co-workers proved that the injection of large amounts of crude liver extract (230, 113), or ascorbic acid (239), caused a considerable decrease of the content of coproporphyrin and uroporphyrin in the excreta.

Rimington (118a), and Rimington, Roets, and Fourie (118i), in their unique studies of porphyria in cattle, isolated from the urine uroporphyrin Type I and small amounts of Type III, as well as coproporphyrin I and small amounts of Type III from urine and feces. One cow excreted daily up to 168 mgm. of uroporphyrin and 90 mgm. of coproporphyrin in the urine, as well as 1,000 mgm. of coproporphyrin in the feces. The ratio of coproporphyrin Type I to Type III in the urine was roughly 28 to 1. Uroporphyrin Type I was isolated from the bones, the bone marrow, spleen, and liver, and was seen spectroscopically in the red blood cells and kidney. It could not be found in the bile nor in the blood plasma. Coproporphyrin Type I was isolated from the bone marrow, spleen, bile, red blood cells, and blood plasma, and was absent in the bones and in the liver.

Fink (33b), and Fink and Hoerburger (33d), reported the isolation of uroporphyrin type I from the bones of pigs with congenital porphyria, and a morphological study is reported by Fikentscher (32a).

Acute porphyria. This condition is a clinical and metabolic entity reviewed by Günther (50a, b). Other cases were reviewed and described by Mason and co-workers (98), and by Vannotti (152b, d). A detailed description of the chemical and clinical findings in this disorder is given by Waldenström in his monograph (155a). A review of the cases in which the types and kinds of porphyrins excreted were determined, and with the chemical findings in three new cases, was published by Mertens (105c).

Cases of acute porphyria do not show light sensitivity and do excrete

large amounts of uroporphyrin type III, chiefly during the acute stage. Waldenström first identified the uroporphyrin excreted as type III compound (155a, b). So-called acute porphyria is in reality a chronic metabolic disorder, and the name was applied because of the fact that large amounts of porphyrins were excreted during the abdominal or paralytic attacks. Waldenström reviewed 179 cases, of which 103 were his own. Since that time others have been reported by Mertens (105c), Dobriner (23a, c), and Turner (148b). Clinical reports were published by Magendanz (95), Bingel (9a), von Drigalski (24), Minnibeck and Strasser (108), Zorn (163), Eldahl (26), Kurt (78), and Geissler (42).

The disease is more common in women than in men, as shown by Waldenström's geographically uniform material. It is familial, and has been observed in three generations. It is inherited as a Mendelian dominant. No single case of "congenital porphyria," was observed in any of the acute porphyria families (155a). The clinical picture varies greatly. The classical symptoms are of three types: 1, abdominal; 2, nervous; 3, mental. All three may appear independently or together. The abdominal symptoms are marked by severe cramp-like pain, vomiting and constipation; often with slight fever, hypertension, and tachycardia. Frequently the acute attacks seem to be associated with menstruation, and cases have been described in which they occurred immediately post partum (155a, 78, 163, 92). The nervous symptoms consist of a symmetrical progressive paresis, sometimes with neuritis. It is often termed Landry's paresis, but Waldenström points out that the classical ascending type of Landry's disease is found only in exceptional cases. The mental cases may present any type of psychic disturbance.

The etiology of acute porphyria is still unknown. Fischer, Waldenström, and Vannotti, suggested that myoglobin might be the source of the uroporphyrin, but this idea has been generally abandoned. Some relation to a disturbed production or breakdown of any of the respiratory pigments is theoretically possible. Waldenström suggested that the disease is a constitutional reaction to toxic factors (155a). No alterations of hematopoiesis are recorded in acute porphyria, but in several cases disturbed liver function, calcium and cholesterol metabolism were observed (1, 101, 155a).

Barker and Estes (5), as well as Vannotti (152b), and others, observed the sudden development of symptoms of Graves' disease in a number of cases. Magendanz's case had an obesity of the Fröhlich type (95); changes in the hypophysis and the adrenals have been described (92); Grünwald (49) reported a virgin who secreted cholestrum, and Harbitz

(55) a man with swelling of the breasts during an attack. Mason et al. (98) reviewed the morphological studies of the disease, and described a lesion of the coeliac ganglion.

In the urine large amounts of uroporphyrin are found. Löeffler (89) isolated a compound with a melting point of 262°C, Fischer and Duesberg (35m, n) of 269°C, Dobriner (23a), of 253°C, Hoerburger and Schulze (64c) of 289°C, and Turner (148b) of 275–278°C. These figures suggest that the pigment excreted is not the uroporphyrin type I found in congenital porphyria. The type I compound of Petry melted at 293°C.

Waldenström (155b), with Fink and Hoerburger (155e), and Waldenström (155a), identified the pigment as uroporphyrin type III. Mertens (105c) confirmed Waldenström's findings and in two cases obtained from a large amount of uroporphyrin type III a small amount of type I. The findings of the authors mentioned suggest that much type III and varying amounts of type I were excreted.

Weiss (158), Waldenström, Fink and Hoerburger (155e), and Waldenström (155a), isolated from the feces coproporphyrin type I, and the last author in one case coproporphyrin type III. Dobriner (23c) isolated coproporphyrin of both types I and III, a finding which Mertens (105c) confirmed in two cases.

The types of porphyrin excreted in acute porphyria are tabulated in Waldenström's (155a) and Merten's (105c) publications. Quantitative determinations were made by Günther (50a), Löeffler (89), Roth (121b), Hoesch and Carrié (65), Kurt (78), and Vannotti (152b).

One patient excreted up to 260,000 micrograms of uroporphyrin daily, and a small amount of coproporphyrin (89). In other instances up to 30,000 micrograms of uroporphyrin are excreted daily in the urine and variable amounts of coproporphyrin in the urine and feces.

Mertens (105c) and Magendanz's (95) patient excreted daily in the urine before a nervous attack 2,000 micrograms: during the attack 50,000 micrograms, and after the attack 11,500 micrograms of uroporphyrin type III, as well as 600 micrograms of unidentified coproporphyrin in the feces. The patient of Mertens (105c) and Bingel (9a) excreted 10,000 micrograms of uroporphyrin type III, 1,100 micrograms of coproporphyrin type III in the urine as well as 300 micrograms of a mixture of coproporphyrin types III and I in the feces. In the serum traces of coproporphyrin and uroporphyrin were present.

Waldenström (155a) and Mertens (105c) could not find any uroporphyrin type III in the bones of patients with acute porphyria. This

is remarkable because the uroporphyrin type I is deposited in relatively large amounts in the bones in congenital porphyria.

The color of the urine during the attacks of acute porphyria is usually red, but in certain cases Waldenström observed a straw-yellow color which darkened considerably after exposure to light. Waldenström (155a), Waldenström and Wendt (155g), and Waldenström with Vahlquist (155f, 150), isolated the chromogens present in the urine of patients with acute porphyria and termed them porphobilin and porphobilinogen. On boiling those substances at an acid reaction, they found small amounts of ether-insoluble porphyrins, possibly uroporphyrin type III. They suggest that the chromogens are two different isomers of dipyrreltetracarbonic acid. A further investigation of the nature of the chromogens in one case was published by Sachs (123).

Chronic porphyria. This term was applied by Günther to certain cases marked by an increased excretion of porphyrin and which could not be classified as either congenital or acute porphyria. Waldenström designated these cases "porphyria cutanea tarda." The skin of the patients is somewhat sensitive to light, and symptoms referable to the intestinal tract are present. The chemical findings, like the clinical manifestations, differ from those of classical cases of congenital and acute porphyria.

Hijmans v. d. Bergh and his co-workers (61a) isolated large amounts of coproporphyrin type III from the excreta of one case. The pigment was identified by Fischer and co-workers (35f). Fischer and Duesberg (35 m), isolated coproporphyrin type III from the urine. Hijmans v. d. Bergh and Grotepass (61c) published a case with renal insufficiency and no copro- or uroporphyrin in the urine. Large amounts of coproporphyrin type I were in the feces and the serum. Grotepass and Delfaque (48e) reported a patient who excreted in the urine small amounts of coproporphyrin type I and III, with no uroporphyrin. From the feces large amounts of coproporphyrin types I and III were isolated, as well as mesoporphyrin number 9 (type III), and protoporphyrin number 9 (type III). No deuteroporphyrin was present. From the case of Gottron and Ellinger (46b), Fischer and Duesberg (35m) isolated uroporphyrin type I and III, and from another case uroporphyrin types I and III, and coproporphyrin type III. From the urine of another patient Hoerburger and Schulze (64c) isolated uroporphyrin type I and coproporphyrin type III. From the urine of the patient of Urbach and Blöch (149), Waldenström isolated uroporphyrin type III (155a). Uroporphyrin types I and III and very small amounts of

unidentified coproporphyrin were obtained from the urine of the case of Wendelberger and Klein (157). The case reported by Dobriner (23c) as chronic porphyria was one of acute porphyria with mental symptoms.

Porphyrins in febrile conditions. Huppert in 1887 reported an increased porphyrin excretion in fever, and Günther (50a, b) reported similar observations and reviewed the literature. Schumm (130c), Lageder (79a), and Tropp and Penew (147b) stated that patients with tuberculosis excreted an increased amount of coproporphyrin (35 to 582 micrograms daily). In no case was the type of porphyrin identified.

In lobar pneumonia Dobriner (23a) isolated coproporphyrin I from both the urine and the feces, and in one case complicated by jaundice a small amount of coproporphyrin III was obtained (23j). Increased amounts of coproporphyrin I were isolated from the urine by Dobriner (23a), Watson (156e), and Vigliani and Libowitzky (153d), from cases of lung abcess. In the febrile attacks of schizophrenic patients Libowitzky and Scheid (84) reported an increased excretion of coproporphyrin I. In induced fever, increased urinary porphyrin values were reported by Carrié (17a), Vannotti (152b), and by Brunsting and co-workers (16b). This increase was not regular, and could not be correlated with the degree of fever. Kapp and Coburn (73b) isolated coproporphyrin III from the urine of patients receiving pyramidon and salicylates for rheumatic fever, and this finding, if confirmed for untreated patients, is of great importance. (Cf. Brownlee's (14) report of pyramidon intoxication in rats.) The evaluation of these data is difficult, but there seems to be no doubt that frequently in febrile states increased amounts of porphyrin are excreted, at least in the urine. In certain instances, furthermore, there is a suggestion that the total output is higher than normal. So far almost no fecal determinations in this type of case have been reported; hence no definite conclusions can be drawn.

Quantitative studies of the porphyrin content of blood in febrile conditions are reported by Hijmans v. d. Bergh and co-workers (61b), who found up to 46 micrograms of porphyrin in 100 cc. of normal blood compared with from 2 to 12 micrograms in normals.

Roets (119) reported a study of the porphyrin metabolism of African cattle with East Coast fever. He isolated from the urine and feces coproporphyrin type III and small amounts of type I. From 100 grams of feces 94 to 104 micrograms of coproporphyrin were obtained

as against 14 to 25 micrograms in normal animals. The ratio of coproporphyrin type I to type III in the urine was 1:31, and in the feces 1:1.5.

Porphyrins in liver disease. Since the early investigators, Salkowski, McMunn, Garrod, and many others, observed an increase of the urinary porphyrins in liver disease, many theories have been advanced concerning this abnormality. We omit a discussion of them since most investigators did not distinguish between coproporphyrin types I and III. In most instances type I is excreted, but in certain cases in which no exogenous intoxication can be demonstrated coproporphyrin III is found. Frequently relatively large amounts of protoporphyrin have been detected in the feces.

The normal liver excretes porphyrins of type I in the urine and the bile, but coproporphyrin III is excreted nearly exclusively through the urine, and to what extent a disturbed liver function may influence the urinary:fecal ratio is unknown. The coproporphyrin excretion in the urine is thought to be a very sensitive indication of hepatic dysfunction, but the authors feel that studies of the urine only, without estimations of the fecal coproporphyrin and determination of the types excreted, do not give adequate information. Brugsch (15a, d) was the first to investigate the urinary:fecal ratio of porphyrin excretion and found it to be between 0.2 and 0.6 under normal conditions. With the methods used he determined coproporphyrin, protoporphyrin, and deuteroporphyrin together in the feces. Localio, Schwartz and Gannon (88), determined the relation of the urinary to the fecal coproporphyrin excretion in normals and in a number of cases of liver dysfunction. In the normals, ratios between 0.3 and 0.6 were observed, and in liver disease from 0.8 to 22.0. It appears that the ratio is of more significance for the diagnosis of liver disease than is the total output, the highest ratios being observed in cases with the most severe liver damage, as judged by clinical evidence.

Watson (156b) isolated coproporphyrin I from the urine of a patient with cirrhosis of the liver due to cinchophen. Dobriner (23a) isolated the same pigment from the urine of 18 patients with catarrhal jaundice, obstructive jaundice, atrophic cirrhosis, and lymphosarcoma of the liver. Vigliani and Libowitsky (153d) isolated coproporphyrin I from a case of cirrhosis.

Dobriner, (23a) isolated coproporphyrin III from the urine of patients with atrophic cirrhosis, hemachromatosis, and melanosarcoma of the liver. From one case of hemachromatosis an unknown porphyrin with a melting point of 222°C was obtained. Vigliani and Libowitzky (153d), isolated coproporphyrin III from a patient with cirrhosis as-

sociated with lues, but the patient had received mercury shortly before the study and this may have contributed to the result.

The isolation of coproporphyrin I was reported by Dobriner (23b), from the feces of patients with atrophic cirrhosis, with catarrhal jaundice and with obstructive jaundice. From the feces of one case of cirrhosis Vigliani and Libowitsky (153d) isolated coproporphyrin I. Dobriner (23b) isolated a mixture of coproporphyrin types I and III from the feces of a case of hemachromatosis which excreted type III in the urine. Zeile and Rau (161a) isolated mesoporphyrin number 9 from the pooled feces of patients with liver disease.

Brugsch (15a, d), Franke (38a), and Dobriner (23b), reported large amounts of porphyrin in the urine of patients with obstructive jaundice. Watson (156f) reported one patient with complete common duct obstruction who excreted no coproporphyrin in the feces, but protoporphyrin and deuteroporphyrin were present. In similar cases with biliary fistulae Vigliani (153f) observed low values for coproporphyrin in the urine, but when no fistula was present the levels were high.

In passive congestion of the liver Thiel (145a, b), Brugsch (15a, d), Dobriner (23a), and Kaunitz (74), observed a moderate increase of urinary coproporphyrin.

Tropp and Penew (147b) reported normal levels of porphyrin in the urine of patients with cholelithiasis and cholecystitis without icterus. In cholangitis Brugsch (15a, b) and Lageder (79a) reported increased levels in the urine (185 to 547 micrograms).

Günther (50a), Thiel (145a, b), Dobriner (23a), Lorente and Scholderer (90), Brugsch (15a, d), Franke (38a), Tropp and Penew (147b), Vigliani (153f), and Kaunitz (74), reported increased rates of excretion of coproporphyrin in the urine of patients with catarrhal jaundice (90 to 408 mcg.) daily. Brugsch (15a, d), Tropp and Penew (147b), and Dobriner (23a), observed during recovery a slow decrease to normal or slightly increased levels. In the blood Vigliani (153e) observed increased levels of the protoporphyrin content of the red blood cells (85 and 111 micrograms). He also found coproporphyrin in the plasma.

In acute and sub-acute yellow atrophy of the liver Günther (50a), Brugsch (15a, d), Franke (38a), and Dobriner (23a), reported excretions of between 180 and 800 micrograms daily.

In hepatic disease due to syphilis Lageder (79a) and Tropp and Penew (147b) reported daily urinary coproporphyrin levels of from 79 to 441 micrograms.

In cirrhosis of the liver Brugsch (15a, d), Lorente and Scholderer

(90), Franke (38a), Kaunitz (74), Lageder (79a), Tropp and Penew (147b) and Dobriner (23a) report 70 to 1250 micrograms of coproporphyrin daily. No suitable quantitative studies of the porphyrin content of the feces in this type of case have been reported.

In lenticular degeneration, Tropp and Penew (147b) observed a normal rate of coproporphyrin excretion in the urine. In a few cases of hemachromatosis Günther (50a), Eppinger (31a, b), Vannotti (152b), and Dobriner (23a), found high levels in the urine. Increased excretion in the urine of patients with metastatic tumors of the liver are reported by Günther (50a), Thiel (145a, b), and Brugsch (15a, d). Daily excretions of from 120 to 615 micrograms are reported by Tropp and Penew (147b), Vigliani (153f), Lorente and Scholderer (90), and Dobriner (23a).

Porphyrins in diseases of the blood. Pernicious anemia. As early as 1897 Taylor (144) found porphyrin in the urine of a patient with pernicious anemia; Günther (50a) reviewed the early studies and reported similar findings. Fischer and Zerweck (34g) found coproporphyrin in both urine and feces.

The coproporphyrin in the urine of patients in relapse was identified as type I in 1937 by Watson (156f), and by Dobriner (23e, h). Watson (156c, f) also identified the coproporphyrin in the feces of three patients as type I. Dobriner (23b), Dobriner and Rhoads (23g), with reports of seven cases, and Vigliani and Libowitsky (153d), confirmed the observation. Watson isolated from the feces of one patient a chloroform-soluble porphyrin of which the methyl ester melted between 189° and 191°C. This was somewhat similar to a porphyrin he isolated from a patient with hemolytic jaundice, and called pseudodeutero-porphyrin A. Increased amounts of protoporphyrin in the feces were found by Watson, Dobriner, Vigliani and Sonzini, and Vigliani and Libowitsky.

Fischer et al. (34m) found coproporphyrin in the bone marrow, and Borst and Königsdörfer (12) observed an increase in the porphyrin content of the megaloblasts and erythroblasts. Watson and Clarke (156h) proved that the increase of protoporphyrin during remission was due to the presence of that pigment in reticulocytes. Vannotti (152b) reports high protoporphyrin levels in the bone marrow with a high coporporphyrin level in one of the cases and a low one in the other. This finding was confirmed by Vigliani and Sonzini (153e), who also found that patients in relapse had a low protoporphyrin content of red cells and plasma, and that the values increased during the

early stages of remission. In relapse traces of coproporphyrin were present in the plasma.

Duesberg (25a), Thiel (145a, b), and Brugsch (15a, c), Lageder (79a), Lorente and Scholderer (90). Vigliani and Sonzini (153e), and Kaunitz (74), made quantitative studies and found somewhat increased levels of coproporphyrin in the urine of certain patients in relapse, whereas in others normal levels were obtained. A decrease in remission was found first by Duesberg (25a), and later by Brugsch (15a), Vannotti (23h), and others. Dobriner with Barker (23e), and with Rhoads (23h), observed three patients in relapse over long periods and found considerable variations (38–169 micrograms) in the daily output. Determinations of the amounts of porphyrins in the feces were made by Brugsch (15a, c), and by Vigliani and Sonzini (153e), but their values are for total porphyrins without quantitative separation of types and therefore cannot be taken as significant. A study of coproporphyrin in the feces was made by Watson (156f). High levels in relapse and low levels during remission were obtained.

The results of the quantitative studies of the urinary and fecal coproporphyrin excretion in relapse and remission in the 3 cases described by Dobriner and Rhoads, together with values for Watson's cases are given in table 6.

Dobriner and Rhoads (23h) employed the rate of excretion of coproporphyrin type I as an index of an orderly type of hematopoietic activity. Hence the findings in pernicious anemia suggested that the disorder was marked by active blood regeneration during relapse and less active during remission.

Hemolytic jaundice. The coproporphyrin excreted in the urine of seven patients with hemolytic jaundice was identified as type I by Dobriner (23a). Two of the cases were of the familial type and five of the acquired. From one familial and one acquired case coproporphyrin type I was isolated both before and after splenectomy. Watson (156e) also isolated coproporphyrin type I from the urine in a case of the acquired disease.

Watson (156e, f), from the feces of 6 patients with acquired and one with familial jaundice, isolated coproporphyrin type I. The same porphyrin was obtained by Dobriner (23a, b, m) from both the urine and the feces of 5 patients. Protoporphyrin was found in increased amounts in the feces by Watson and by Dobriner. Watson isolated and described the properties of a chloroform-soluble porphyrin obtained from the deuteroporphyrin fraction, and which could not be identified.

The melting point of the ester was between 202° and 203°C. He called this compound pseudo-deuteroporphyrin A; it may be identical with that described by Schumm (130f) as saproporphyrin.

The literature contains reports of variable results of quantitative studies of coproporphyrin in the urine of patients with hemolytic jaundice. Günther (50a), Fischer and Zerweck (34g), Duesberg (25a), Lageder (79a), Vigliani and Sonzini (153e), and Vannotti (152b), found the content generally low. Dobriner (23a), in four cases of acquired and two cases of familial hemolytic jaundice, reported levels higher than normal. In one patient with a severe hemolytic crisis of the ac-

TABLE 6

The average daily excretion of coproporphyrin I and of urobilinogen in relapse and remission of pernicious anemia and hemolytic jaundice

CASE NUMBER	COPROPORPHYRIN TYPE I (MCG.)						UROBILINOGEN (MGM.)		
	Urine		Feces		Total				
	Relapse	Remission	Relapse	Remission	Relapse	Remission	Relapse	Remission	Reference
1	158	42	474	130	632	172	442	93	(23g)
2	138	66	474	282	612	305	900	150	(23g)
3	133	95	345	105	478	200	478	262	(23g)
4			520	260					(156b)
5			570	240					(156b)
	a	b	a	b	a	b	a	b	
*	318	81	401	290	718	371	407	73	(23m)
Normals.....	87–123		205–274		306–376		Up to 150		

a = before splenectomy.
b = after splenectomy.
* Case of hemolytic jaundice.

quired disease, as much as 2,500 micrograms daily of urinary coproporphyrin were excreted, whereas in a previous period of observation no more than 800 micrograms per day had been obtained. In another unpublished case the average daily excretion over a 15 day period was 211 micrograms in the urine and 441 in the feces. Watson found 1,020 micrograms in the feces of one patient (156f).

On the whole, investigators seem now to agree that the total porphyrin excretion in both acquired and hemolytic jaundice is definitely increased. After splenectomy the output decreases both in urine and in feces, as shown by Dobriner (23a, m) and Watson (156f). Dobriner and co-

workers (23m) called attention to the association of the increased orderly type of hematopoietic activity in this disease with an increased formation of coproporphyrin type I.

Porphyrins in hemorrhage. Duesberg (25a) and others produced anemia in rabbits by hemorrhage as well as by distilled water and chemicals. The rate of porphyrin excretion in the urine was measured and no increase observed. More prolonged studies with determinations of fecal levels should have been made. Dobriner, and Dobriner and Rhoads, followed the urinary and fecal coproporphyrin levels in dogs after hemorrhage (23g) and with hemolysis from phenylhydrazin (23d). Coproporphyrin type I was excreted in both feces and urine in the control periods. Eight to twelve days after treatment, and when blood regeneration was well established, a marked increase in the rate of excretion of the same compound was obtained. In figure 5 the results of the experiment with phenylhydrazin are shown. A similar observation was reported by Dobriner et al. (23n) in a case of polycythemia in which phenylhydrazin was administered.

These experiments were made to demonstrate that type I and type III porphyrins are produced simultaneously as the products of the same synthesis.

Thiel (145a, b), Brugsch (15a, c), and Vannotti (152b), observed in some patients with hemorrhage into the intestine an increased rate of porphyrin excretion in the urine (143 to 175 micrograms daily), but in other cases found normal levels. Following venesection, phenylhydrazin administration, and thrombocytopenic purpura, normal urinary levels were observed. Langen and Grotepass (81a, b) followed the blood prophyrin levels (protoporphyrin) in animals after hemorrhage and chemical hemolysis. They found increased levels as regeneration proceeded.

Porphyrins in aplastic anemia. Brugsch (15a, c) in a patient with aplastic anemia observed low, and Vannotti (152b) in a similar case, increased urinary porphyrin levels. Dobriner, Rhoads and Hummel (23i), in six cases of aplastic anemia observed in four the excretion of coproporphyrin I and III in the urine and feces. In two more coproporphyrin III could not be identified by melting point determinations, but its presence was suggested by the chemical data. The patients' porphyrin excretion was followed for long periods, and the influence on it of vitamin A and vitamin C, as well as liver extract, was investigated. The data for the control periods is given in table 7. Because of the finding of type III coproporphyrin, Dobriner, Rhoads,

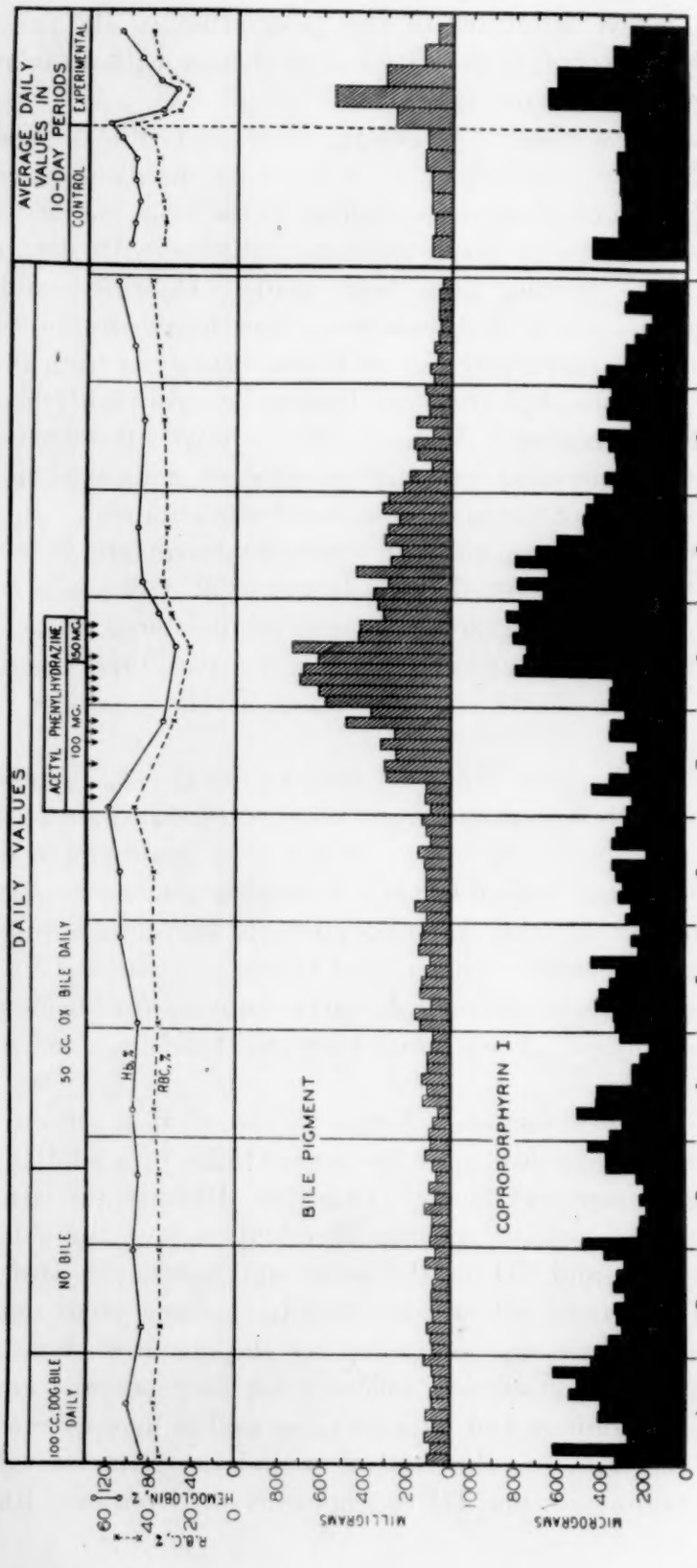

Fig. 5. A chart showing the rate of excretion of bile pigment and coproporphyrin I by a dog with hemolysis by phenylhydrazin. The high coproporphyrin values in the first period are due to the coproporphyrin present in the administered dog bile.

and Hummel suggest that the disease may be due to the action of toxic factors which result in the excretion of an abnormal porphyrin, as is the case in poisoning by lead and aromatic amines.

In sickle-cell anemia Dobriner, Rhoads, and Erf (23g) observed a marked increase of the total porphyrin excretion by a negro child. The average daily level was 470 micrograms for an 18-day observation period, and from the urine and feces coproporphyrin I was isolated and identified.

In erythroblastic anemia in a 4-year old child, Tropp and Penew (147b) observed daily urinary coproporphyrin levels of between 108

TABLE 7

The average daily total excretion of coproporphyrins by patients with aplastic anemia

The rate of excretion of coproporphyrin and urobilinogen, the blood levels, and the type of the bone marrow in the control period as well as the type of coproporphyrin excreted

CASE NUMBER	CONTROL PERIOD	AVERAGE DAILY COPROPORPHYRIN EXCRETION			AVERAGE DAILY UROBILINOGEN EXCRETION	RED BLOOD CELLS	HEMOGLOBIN	WHITE BLOOD CELLS	BONE MARROW	TYPE OF COPROPORPHYRIN EXCRETED
		Urine	Feces	Total						
	days	*micrograms*	*micrograms*	*micrograms*	*mgm.*	*millions*	*per cent*			
1. H. D.	9	89	122	210	83	1.100	21	1000	Hypoplastic	I (and III?)
2. C. S.	12	65	178	243	133	1.230	35	800	Hypoplastic	I and III
3. F. Y.	9	220	197	417	99	2.400	70	2250	Immature	I (and III?)
4. G. M.	12	212	280	492	370-450	1.110	22	950	Normally cellular	I and III
5. W. G.	9	550	428	978	273	1.42	27	1300	Hyperplastic	I and III
6. A. C.	21	201	493	694	128-165	1.440	39	2500	Hypoplastic	I and III
Normals....	9	87-123	205-274	306-376	150					I

and 256 micrograms, and after splenectomy normal levels in the urine were found.

Leukemia. Duesberg (25a) and Thiel (145a, b) found no increase of coproporphyrin in the urine in myeloid leukemia. Brugsch (15a, c) reported normal values in one case, and in another a daily excretion of 186 micrograms. Lorente and Scholderer (90), as well as Vannotti (152b), observed an increased urinary porphyrin excretion (83 to 221 micrograms) daily. No studies of the porphyrin content of the feces in leukemia have been reported.

Hodgkin's Disease. In cases of Hodgkin's disease Duesberg (25a)

and Brugsch (15c) observed a normal rate of coproporphyrin excretion in the urine. Dobriner (23a, b) in severe febrile cases shortly before death observed daily excretion rates of 200 to 600 micrograms of coproporphyrin in the urine. Coproporphyrin I was identified from the urine of 5 patients and from the urine and feces of one.

Polycythemia. In a case of polycythemia rubra (Vaquez) with hypoplasia of the red bone marrow, Vannotti (152b) found a daily urinary porphyrin excretion of 273 to 398 micrograms, with a corresponding increase of the coproporphyrin in the plasma and of porphyrin (protoporphyrin?) in the red blood cells. Brugsch (15c) found 93 micrograms in the urine of one patient.

In a case of polycythemia vera Dobriner and co-workers (23m) found in the urine and in the feces, a total average excretion of 371 micrograms per day over a 6-day period; this value may be considered in the high normal range for the age of the patient. The porphyrin was identified as coproporphyrin I in both urine and feces. Günther (50a), Vannotti (152b), and Carrié (17a), found normal levels in the urine of similar patients, and Vannotti (152b), no increase of the plasma porphyrins. Carrié (17a) observed a patient under x-ray treatment whose urine showed an increase of coproporphyrin as anemia developed.

Deficiency diseases. Pellagra. The association of light sensitivity with increased porphyrin excretion has directed attention to the porphyrin metabolism in pellagra. Massa (99) saw increased porphyrinuria in endemic pellagrins in Italy; a finding which was confirmed by Bassi (6), by Ellinger and Dojmi (29), Beckh, Ellinger, and Spies (7). Spies and co-workers (137) studied patients with alcoholic and endemic pellagra and stated that they excreted increased amounts of porphyrin. The methods employed by Spies et al. are discussed by Dobriner and Rhoads (23k), and Watson (156g). Dobriner (23b) isolated coproporphyrin I from the urine and feces of a case of alcoholic pellagra in 1937, and the findings strongly suggested the presence of coproporphyrin III. Dobriner, Strain, and Localio (23n) reported studies of the urinary and fecal excretion in alcoholic pellagra before and after therapy. In the urine during the control period 254 mcg. coproporphyrin were excreted daily, and the total urinary and fecal output during the control period of 6 days was 897 micrograms with large amounts of protoporphyrin and deuteroporphyrin in the feces, whereas after treatment the total amounts of coproporphyrin dropped to normal. Spies and co-workers (137) mention the isolation of coproporphyrin III from the urine of 2 cases but chemical proof of its type was not presented. Wat-

son (156g) reports studies of the porphyrin excretion of 3 alcoholic pellagrins during a 10 day observation period and while under treatment with nicotinic acid. In all 3 he observed high levels in the urine (up to 425 micrograms) during the control period, and a sharp decrease after therapy. He isolated and identified coproporphyrin type III from one case.

The effect of coproporphyrin types I and III in sensitizing to light is discussed elsewhere; but the rôle of these substances in causing the dermatitis of pellagra is still unclear. In view of the heightened excretion of porphyrins in alcoholism and the excretion of coproporphyrin III in the intoxications associated with damage to the liver, a dysfunction of that organ in pellagra is suggested. It would be interesting to know precisely the facts concerning the porphyrin metabolism in cases of endemic pellagra without alcoholism. Brunsting, Brugsch, and O'Leary (16b) reported a normal urinary output in two cases of secondary pellagra.

Roncoroni (120) observed an increased porphyrin excretion by guinea pigs which had been fed a corn diet to produce experimental pellagra.

Sprue. Vannotti (152b) reported studies of three cases of sprue, in two of which the urinary coproporphyrin excretion was normal. In the third there was an increased output in the urine (100–170 micrograms). A large amount of a mixture of coproporphyrin and deuteroporphyrin was found in the feces. He administered riboflavin and liver extract and found that the rate of excretion decreased considerably.

Porphyrins in diseases of the skin. The association of increased rates of excretion of porphyrins of different types with disorders marked by light sensitivity has been referred to previously. A number of observations are recorded concerning various rates of urinary porphyrin excretion by patients with disorders of the skin. In many instances the methods employed are not stated, and because of this fact the results are difficult to evaluate.

Günther (50a) has reviewed the earlier literature. Goeckermann and co-workers (45) found increased urinary levels in the case of eczema, and Marquardt (97) reported similar findings in salvarsan dermatitis, eczema and other conditions. Hübner (67) reported levels as high as 600 micrograms daily of urinary porphyrins in 8 of 32 cases of erythema multiforme, and claims to have found uroporphyrin as well as coproporphyrin. His methods, however, are open to question. Scolari (131) found an increased excretion in erythroderma and other skin affections, and Ludy and Corson (91) an increase in lupus erythematosis,

an observation which could not be confirmed by Brunsting and his co-workers (16b). The writers had an opportunity recently to examine 24-hour collections of urine from cases of this disorder; in three no increase of porphyrin content was observed, while in one a high level was found shortly before death. McFarland and Strain (104), and Tropp (147c), recently reported studies of a series of cases of skin disease. In a case of xeroderma pigmentosa unequivocally high levels were found. Zoon (162) saw in the same condition a high urinary and high blood porphyrin levels. Brunsting and co-workers (16b) reported studies of the urinary porphyrin excretion in a large series of cases of diseases of the skin, and in no instance was uroporphyrin found. High rates of urinary excretion were found in cases of exfoliative dermatitis and in extensive bacterial ulcerations of the skin, in widespread burns, erythroderma, Hodgkin's disease of skin, erythema multiforme and pemphigus vulgaris. Of nine cases of eczema solare and urticaria solare, only two were found to have a slight increase of porphyrin output. In one case of pityriasis rosea the fecal porphyrin excretion was high and the urinary output normal. The only other high fecal values were observed in cases of salvarsan dermatitis.

Porphyrins in mental diseases. Very few reports are available concerning the porphyrin excretion in mental disturbances. Brugsch (15e) did not find an increase of the porphyrin output in the urine, but in two cases of excited schizophrenic patients he found levels of 181 and 184 micrograms. Scheid and Libowitsky (84) reported an increased coproporphyrin I production and excretion during the febrile attacks in schizophrenic patients.

Porphyrins in toxic states. Lead. One of the earliest examples of toxic porphyrinuria was lead poisoning, described by Garrod (41b) in 1892, and by Stockvis (139) in 1895. The literature up to 1925 has been discussed in detail by Günther (50a, b) and needs no further consideration.

All agree that coproporphyrin is present in increased amounts in the urine of patients with lead poisoning. Grotepass (48a, d) identified the pigment as coproporphyrin type III, an important observation since verified by Watson (156e), Vigliani and Waldenström (153c), and Mertens (105a). Dobriner (23s) isolated and identified a large amount of the type III compound and a small amount of type I in the urine of several patients with acute symptoms. From the feces Watson (156f), and Vigliani and Libowitzky (153d), isolated coproporphyrin Type I. Dobriner (23g) however, obtained a small amount of copro-

porphyrin type III as well as coproporphyrin type I. Vigliani and co-workers (153g), Watson, Dobriner and Mertens, found protoporphyrin in the feces. Both feces and urine have been studied in a few instances. Watson (156f), as well as Vigliani and Libowitzky (153d), found coproporphyrin III in the urine and coproporphyrin I in the feces. Dobriner (23b, r) obtained large amounts of coproporphyrin III and small amounts of type I from the urine. In the feces, however, the order was reversed.

The porphyrin content of plasma and erythrocytes in lead poisoning was studied by Vigliani and Angeleri (153b, g), who found increased amounts of protoporphyrin and in certain instances traces of coproporphyrin. Günther (50a) reviewed the quantitative studies. The patients of Grotepass (48d), Schreus and Carrie (129c), Vigliani with Sasso et al. (153a, g), Vigliani and Libowitzky (153d), Roth (121a), Vigliani and Waldenström (153c), Frank and Litzner (38c), Vannotti (152b), and Mertens (105a) excreted between 630 and 14,800 micrograms daily in the urine.

Very few quantitative determinations of coproporphyrin in the feces of patients with lead poisoning have been made with adequate methods. Mertens (105a) studied one case and obtained normal values. Dobriner and Strain (23r) observed several cases during and after attacks of acute colic. In both periods increased amounts of coproporphyrin and protoporphyrin, as well as small amounts of deuteroporphyrin, were excreted.

Increased light sensitivity has been reported by Carrié (17a), Roth (121a), and Vannotti (152b). Hijmans v.d.Bergh and co-workers (61b) administered lead salts to human beings, and observed increased urinary porphyrin excretion after from 2 to 12 days. Vigliani et al. (153a, g) in similar studies found the urinary porphyrin excretion in the control period averaged 51 micrograms per day; on the fourth day of poisoning it rose to 91 micrograms, and at the end of 9 days had reached 522 micrograms per day. After treatment was discontinued the excretion decreased slowly and reached normal levels after four weeks. No determinations of coproporphyrin levels in the feces were made. It has been suggested by some investigators that the increased porphyrin excretion which marks acute lead poisoning may be of diagnostic value (Günther, 50a; Franke and Litzner, 38c). Roth (121a) did not see any effect of liver therapy on the porphyrin excretion, but Schreus and Carrié (129c) thought that they influenced it by Campolon injections.

In 1895 Stockvis (139) produced by lead poisoning in rabbits an increased excretion of urinary porphyrins, and the experiments have been repeatedly confirmed. The earlier literature is reviewed in the publications of Günther (50a, b), Liebig (86), and Duesberg (25a). Fischer and Duesberg (35m) isolated coproporphyrin III from the urine of rabbits with lead poisoning. No increase of coproporphyrin was found in the feces. Waldenström (155a) confirmed the findings regarding coproporphyrin type III, but isolated coproporphyrin I from the urine of one animal. Uroporphyrin could not be detected, but an exceedingly small amount of an ether-insoluble, but ethyl acetate-soluble, porphyrin (uroporphyrin III?) was found. In the bile Waldenström found coproporphyrin and protoporphyrin.

The bone marrow in experimental lead poisoning was studied by Liebig (86), Duesberg (25a), Emminger and Battestini (30a), and Vannotti (152b). Liebig extracted relatively large amounts of porphyrin of an unknown type and Waldenström (155a) obtained copro porphyrin. The latter states that free protoporphyrin was not present. Emminger and Battestini (30a) obtained evidence that by the administration intravenously of calcium chloride to animals poisoned with lead, the increased excretion of porphyrin in the urine could be restored to normal and the bones were no longer fluorescent. If lead and calcium chloride were given simultaneously no porphyrinuria developed, nor could evidence of increased erythropoiesis in the marrow be obtained. It is unfortunate that no determinations of fecal porphyrins were made in this study.

Sulfonal. Porphyrinuria in sulfonal poisoning was reviewed by Günther (50a, b), who reported 66 cases, of which 92 per cent were female. The same author reported 11 patients with trional poisoning, of which 9 were females. The symptoms are in many respects similar to those of acute porphyria. Ellinger and Riesser (27) isolated from the urine of a patient with trional poisoning a uroporphyrin melting between 255°C and 257°C, a finding which suggested that the compound was of type III. Dobriner (23a) from a case of sulfonal poisoning isolated a uroporphyrin melting at 280°C, an indication that it was type I with a small amount of type III. Germuth (43) and Brugsch (15a, l) proved that sulfonal and trional increased the excretion of porphyrins by patients, and the former felt that the substitution of the ethyl radical for the methyl group increased the porphyrin production.

Stockvis (139) produced an increased excretion of porphyrin in the urine by feeding sulfonal to rabbits and to dogs. A thorough investi-

gation of the subject was made by Neubauer (112). Duesberg (25a) could induce porphyrinuria in rabbits, as could Laubendender and Monden (82) and Polson (115). Fischer and Zerweck were not able to do so, however. Fischer and Duesberg (35m) found uroporphyrin, but no coproporphyrin in the urine of rabbits with sulfonal poisoning, and from the feces of the animals a porphyrin with a melting point of 188°C could be isolated. The same compound was isolated in lead poisoning, and in much smaller amounts, from normal rabbit feces. Waldenström and Wendt (155g) poisoned rabbits with dimethylsulfondimethylmethan and caused a temporary, inconstant, increase of urinary porphyrin. This was identified by Waldenström as coproporphyrin type III. In two animals a suggestion of an ether-insoluble porphyrin (uroporphyrin?) was found.

Salvarsan. Cavina (18), Schreus (129a), Carrié (17a), and Scolari (131) investigated patients receiving salvarsan and found an increase in amount of coproporphyrin in the urine. Hoerburger and Fink (64b) identified it as a type III compound. Marquardt (97), Brugsch and O'Leary (16b), observed increased levels of coproporphyrin in the urine in some cases of salvarsan dermatitis (199 to 684 micrograms daily). The latter authors reported a fecal excretion of 1328 micrograms of coproporphyrin daily in one case, but in three others no increase was present.

Alcohol. Franke and Fikentscher (38b) and Franke (38a) investigated the influence of the administration of alcohol to human beings on the excretion of porphyrin in the urine. They found that moderate amounts increased the output to double the normal level. Brugsch (15e) and Brugsch and Keys (15g) found that in chronic alcoholism the porphyrin output in the urine was greatly increased (500 to 2,000 micrograms daily), whereas the fecal excretion was reported to be low. Coproporphyrin type III was isolated in one instance, and a small amount of coproporphyrin type I was present also. From another case only the type I compound was isolated. The excretion of type III compound in alcoholism should be kept in mind in view of the similar finding in alcoholic pellagra, and in certain cases of liver disease.

Phosphorus. In phosphorus poisoning in animals a great increase of urinary porphyrin excretion was observed by Lorente and Scholderer (90), Perutz (113), and Thomas (146b).

Selenium. Halter (52) published a report of a patient with selenium poisoning who showed an increased excretion of porphyrin in the urine. Vannotti (152b, l) reported a case of mercury poisoning with the ex-

cretion of uroporphyrin and coproporphyrin type I in the urine. The coproporphyrin output was 4,000 to 12,000 micrograms daily. The patient showed signs of light sensitivity and after two years still excreted 200 to 1300 micrograms of coproporphyrin.

Dibenzanthracene. Dobriner and Rhoads (23j) observed increased urinary porphyrin levels in rabbits treated with 1,2,5,6,dibenzanthracene. Coproporphyrin types I and III were excreted in nearly equal amounts. Normal rabbits excrete small amounts of coproporphyrin III.

Barbiturates. The influence of barbiturates on the excretion of porphyrins was reviewed by Günther (50a, b) but he reports no clear-cut results. Brugsch (15e) studied the same subject in psychopathic patients and observed a doubtful increase in rare instances. Miscellaneous observations of increased porphyrin excretion in poisoning by sedatives are reported by Haxthausen (58), Duesberg (25c), and Fischer and Duesberg (35m). The latter isolated uroporphyrin I in a case of intoxication of unknown nature. Laubender and Monden (82), in an experimental study found no increase in the urinary porphyrin excretion by rabbits fed barbiturates.

Sulfonamides. Brunsting (16a) and Brunsting and co-workers (16b) studied several patients with dermatitis and photosensitivity resulting from treatment with sulfanilamide, and in two found increased amounts of porphyrin in the urine. In a detailed study Rimington and Hemmings (118g) observed rates of coproporphyrin excretion daily in the urine up to 463 micrograms. They established the fact that coproporphyrin types I and III were excreted in equal amounts. The feces were not investigated. Silver and Elliot (134) demonstrated an increased amount of porphyrin in the urine of patients receiving sulfanilamide, but McFarland and Strain (104) did not find any increase.

Rimington and Hemmings (118g) reported an increased excretion of coproporphyrin in the urine and feces of rats treated with sulfanilamide. They identified coproporphyrin III and obtained suggestive evidence of the presence of traces of type I in the urine. In the feces a mixture of types I and III was present. The treated animals excreted $2\frac{1}{2}$ to 10 times as much porphyrin as normals. The protoporphyrin present was identified as a type III compound and careful investigation revealed no type I. Wien (159) repeated and confirmed Rimington's experiments, but found no porphyrinuria after sulfapyridine administration.

Rimington and Hemmings (118f, h) reported extensive animal experiments concerning the porphyrinuric action of 27 compounds re-

lated to sulfanilamide and other aromatic amines. They observed an increased excretion of coproporphyrin III in those cases which showed methemoglobin formation in the blood. Brownlee (14) isolated coproporphyrin III and small amounts of coproporphyrin I from the urine of rats treated with acetanilid, phenacetin, phenazone, amidopyrine, aspirin and p-aminophenol. None of the authors mentioned describes the type of porphyrin excreted by normal rats. Since Dobriner and Rhoads (23j) observed the excretion of coproporphyrin III in the urine of normal rabbits it seems to be of the utmost importance to know the type of coproporphyrin excreted by normal rats. In illuminating gas intoxication Tropp and Penew (147b) found a large amount of coproporphyrin in the urine. The type was not identified.

Coproporphyrin type III theoretically can be produced by abnormal breakdown or synthesis of type III porphyrins contained in the respiratory pigments. It is produced in cases of intoxication by lead and other substances, as well as in certain diseases. Hijmans v.d. Bergh (61b) has shown that coproporphyrin is formed by the liver from protoporphyrin. Recently Rimington and Hemmings (118f, h) and independently Brownlee (14) advanced the idea that coproporphyrin type III may be produced after the formation of methemoglobin, "which, when once formed, is degraded, in part at least, by a mechanism which leads ultimately not to bile pigment but to porphyrins. Hematin may possibly represent one of the intermediate stages in this transformation" (Rimington); or, as Brownlee says, "where hemoglobin is oxidized to methemoglobin, the normal conversion into bilirubin cannot occur but is replaced by degradation to coproporphyrin III."

REFERENCES

(1) Althausen, T. L. Klin. Wchnschr. **10:** 1016, 1931.
(2) Anson, M. L. and A. E. Mirsky. Physiol. Rev. **10:** 506, 1930.
(3) Ashby, H. T. Quart. J. Med. **19:** 375, 1926.
(4a) Barkan, G. and O. Schales. Ztschr. f. physiol. Chem. **248:** 96, 1937.
(b) Barkan, G. Klin. Wchnschr. **16:** 1265, 1937.
(5) Barker, L. F. and W. L. Estes, Jr. J. A. M. A. **59:** 718, 1912.
(6) Bassi, U. Clin. med. ital. **65:** 241, 1934.
(7) Beckh, W., P. Ellinger and T. D. Spies. Quart. J. Med. **30:** 305, 1937.
(8) Bingold, K. Deutsch. Arch. f. klin. Med. **177:** 230, 1935.
(9a) Bingel, A. Fortschr. d. Neurol. **9:** 265, 1937.
(b) Bingel, A. Ztschr. f. d. ges. Neurol. u. Psychiat. **158:** 79, 1937.
(10a) Blum, H. F. Physiol. Rev. **12:** 23, 1932.
(b) Blum, H. F. and N. Pace. Brit. J. Dermat. and Syph. **49:** 465, 1937.
(c) Blum, H. F. and I. C. Schumacher. Ann. Int. Med. **11:** 548, 1937.

(11a) Boas, I. Arch. f. Verdauungskr. **53**: 87, 1933.
(b) Boas, I. Klin. Wchnschr. **12**: 589, 1933.
(12) Borst, M. and H. Königsdörffer. Untersuchungen über Porphyrie. S. Hirzel, Leipzig, 1929.
(13) Boyd, M. J. J. Biol. Chem. **103**: 249, 1933.
(14) Brownlee, G. Biochem. J. **33**: 697, 1939.
(15a) Brugsch, J. T. Ergebn. d. inn. Med. u. Kinderh. **51**: 86, 1936.
(b) Brugsch, J. T. Ztschr. f. d. ges. exper. Med. **95**: 471, 1935.
(c) Brugsch, J. T. Ztschr. f. d. ges. exper. Med. **95**: 482, 1935.
(d) Brugsch, J. T. Ztschr. f. d. ges. exper. Med. **95**: 493, 1935.
(e) Brugsch, J. T. Ztschr. f. d. ges. exper. Med. **99**: 585, 1936.
(f) Brugsch, J. T. Ztschr. f. d. ges. exper. Med. **103**: 503, 1938.
(g) Brugsch, J. T. and A. Keys. Proc. Staff Meet. Mayo Clinic **12**: 609, 1937.
(16a) Brunsting, L. A. Proc. Staff Meet. Mayo Clinic **12**: 614, 1937.
(b) Brunsting, L. A., J. T. Brugsch and P. A. O'Leary. Arch. Dermat. and Syph. **39**: 294, 1939.
(17a) Carrié, C. Die Porphyrine. G. Thieme, Liepzig, 1936.
(b) Carrié, C. and L. Herold. Arch. f. Gynäk. **158**: 54, 1934.
(c) Carrié, C. and A. S. v. Mallinckrodt. Arch. f. Dermat. u. Syph. **170**: 521, 1934.
(18) Cavina, C. Gior. ital. d. mal. ven. **58**: 263, 1917.
(19) Conant, J. B. and S. E. Kamerling. J. Am. Chem. Soc. **53**: 3522, 1931.
(20) Coulter, C. B. and F. M. Stone. Proc. Soc. Exper. Biol. and Med. **38**: 423, 1938.
(21) Derrien, E. and P. Cristol. Compt. rend. Soc. de biol. **103**: 126, 1930.
(22) Dhéré, C. La fluorescence en biochimie. Presses univ. de France, Paris, 1937.
(23a) Dobriner, K. J. Biol. Chem. **113**: 1, 1936.
(b) Dobriner, K. J. Biol. Chem. **120**: 115, 1937.
(c) Dobriner, K. Proc. Soc. Exper. Biol. and Med. **35**: 175, 1936.
(d) Dobriner, K. Proc. Soc. Exper. Biol. and Med. **36**: 757, 1937.
(e) Dobriner, K. and W. H. Barker. Proc. Soc. Exper. Biol. and Med. **36**: 864, 1937.
(f) Dobriner, K., S. A. Localio and W. H. Strain. J. Biol. Chem. **114**: xxvi, 1936.
(g) Dobriner, K. and C. P. Rhoads. J. Clin. Investigation **17**: 105, 1938.
(h) Dobriner, K. and C. P. Rhoads. J. Clin. Investigation **17**: 95, 1938.
(i) Dobriner, K., C. P. Rhoads and L. E. Hummel. J. Clin. Investigation **17**: 125, 1938.
(j) Dobriner, K. and C. P. Rhoads. Unpublished.
(k) Dobriner, K. and C. P. Rhoads. New England J. Med. **219**: 1027, 1938.
(l) Dobriner, K., W. H. Strain and S. A. Localio. Proc. Soc. Exper. Biol. and Med. **36**: 752, 1937.
(m) Dobriner, K., W. H. Strain, S. A. Localio, H. Keutmann and D. I. Stephens. Proc. Soc. Exper. Biol. and Med. **36**: 755, 1937.
(n) Dobriner, K., W. H. Strain and S. A. Localio. Proc. Soc. Exper. Biol. and Med. **38**: 748, 1938.

(o) Dobriner, K., W. H. Strain, H. Guild and S. A. Localio. J. Clin. Investigation **17**: 761, 1938.
(p) Dobriner, K., C. P. Rhoads and L. A. Erf. Unpublished observations.
(q) Dobriner, K. and H. Guild. Unpublished observations.
(r) Dobriner, K. and W. H. Strain. Unpublished observations.
(24) v. Drigalski, W. Klin. Wchnschr. **16**: 1779, 1937.
(25a) Duesberg, R. Arch. f. exper. Path. u. Pharmakol. **162**: 249, 1931.
(b) Duesberg, R. Arch. f. exper. Path. u. Pharmakol. **162**: 280, 1931.
(c) Duesberg, R. München. med. Wchnschr. **79**: 1821, 1932.
(26) Eldahl, A. Acta med. Scandinav. **97**: 415, 1938.
(27) Ellinger, A. and O. Riesser. Zeitschr. f. physiol. Chem. **98**: 1, 1916.
(28) Ellinger, F. Sonderbände z. Strahlentherapie **20**: 1935.
(29) Ellinger, P. and L. Dojmi. Chem. and Indust. **13**: 507, 1935.
(30a) Emminger, E. and O. Battistini. Virchow's Arch. **290**: 492, 1933.
(b) Emminger, E. and B. Büchele. Virchow's Arch. **295**: 46, 1935.
(31a) Eppinger, H. Die hepato-lienalen Erkrankungen. J. Springer, Berlin, 1920.
(b) Eppinger, H. Die Leberkrankheiten. J. Springer, Berlin, 1937.
(32a) Fikentscher, R. Virchow's Arch. **279**: 731, 1931.
(b) Fikentscher, R. Biochem. Ztschr. **249**: 257, 1932.
(c) Fikentscher, R. Arch. f. Gynäk. **154**: 129, 1933.
(d) Fikentscher, R. Ztschr. f. Geburtsh. u. Gynäk. **111**: 164, 1935.
(e) Fikentscher, R. Ztschr. f. Geburtsh. u. Gynäk. **111**: 210, 1935.
(f) Fikentscher, R. Klin. Wchnschr. **14**: 569, 1935.
(g) Fikentscher, R., H. Fink and E. Emminger. Virchow's Arch. **287**: 764, 1933.
(33a) Fink, H. Biochem. Ztschr. **211**: 65, 1929.
(b) Fink, H. Ztschr. f. physiol. Chem. **197**: 193, 1931.
(c) Fink, H. Ber. d. deutsch. chem. Gesellsch. **70**: 1477, 1937.
(d) Fink, H. and W. Hoerburger. Ztschr. f. physiol. Chem. **202**: 8, 1931.
(e) Fink, H. and W. Hoerburger. Ztschr. f. physiol. Chem. **218**: 181, 1933.
(f) Fink, H. and W. Hoerburger. Ztschr. f. physiol. Chem. **232**: 28, 1935.
(g) Fink, H. and W. Hoerburger. Naturwissenschaften **22**: 292, 1934.
(34) Fischer, H. and H. Orth. Die Chemie des Pyrrols. Akad. Verlagsgesellsch., Leipzig, 1937.
(a) Fischer, H. Ztschr. f. physiol. Chem. **95**: 34, 1915.
(b) Fischer, H. Ztschr. f. physiol. Chem. **96**: 148, 1915.
(c) Fischer, H. Ztschr. f. physiol. Chem. **97**: 109, 1916.
(d) Fischer, H. Ztschr. f. physiol. Chem. **259**: Supp. 1, 1939.
(e) Fischer, H. In: Handbuch d. biol. Arbeitsmethoden (Abderhalden), Abt. I, Teil 11, 1926.
(f) Fischer, H. and K. Schneller. Ztschr. f. physiol. Chem. **135**: 253, 1924.
(g) Fischer, H. and W. Zerweck. Ztschr. f. physiol. Chem. **132**: 12, 1924.
(h) Fischer, H. and W. Zerweck. Ztschr. f. physiol. Chem. **137**: 176, 1924.
(i) Fischer, H. and J. Hilger. Ztschr. f. physiol. Chem. **138**: 49, 1924.
(j) Fischer, H. and F. Kögl. Ztschr. f. physiol. Chem. **138**: 262, 1924.
(k) Fischer, H. and F. Lindner. Ztschr. f. physiol. Chem. **145**: 202, 1925.
(l) Fischer, H. and F. Lindner. Ztschr. f. physiol. Chem. **161**: 18, 1926.

(m) FISCHER, H., H. HILMER, F. LINDNER AND B. PÜTZER. Ztschr. f. physiol. Chem. **150**: 44, 1925.
(n) FISCHER, H. AND H. FINK. Ztschr. f. physiol. Chem. **150**: 243, 1925.
(o) FISCHER, H. AND H. HILMER. Ztschr. f. physiol. Chem. **153**: 167, 1926.
(35a) FISCHER, H. AND A. TREIBS. Tabulae biologicae **3**: 339, 1926.
(b) FISCHER, H. AND B. PÜTZER. Ztschr. f. physiol. Chem. **154**: 39, 1926.
(c) FISCHER, H. AND F. SCHWERDTEL. Ztschr. f. physiol. Chem. **159**: 120, 1926.
(d) FISCHER, H. AND F. LINDNER. Ztschr. f. physiol. Chem. **161**: 1, 1926.
(e) FISCHER, H. AND P. HEISEL. J. v. Liebig's Ann. d. Chem. **457**: 83, 1927.
(f) FISCHER, H., K. PLATZ AND K. MORGENROTH. Ztschr. f. physiol. Chem. **182**: 265, 1929.
(g) FISCHER, H. Verhandl. d. deutsch. Gesellsch. f. inn. Med. **45**: 7, 1933.
(h) FISCHER, H. AND K. ZEILE. J. v. Liebig's Ann. d. Chem. **468**: 98, 1929.
(i) FISCHER, H., A. TREIBS AND K. ZEILE. Ztschr. f. physiol. Chem. **193**: 138, 1930.
(j) FISCHER, H. AND J. HIRNEIS. Ztschr. f. physiol. Chem. **196**: 155, 1931.
(k) FISCHER, H. AND K. JORDAN. Ztschr. f. physiol. Chem. **190**: 75, 1930.
(l) FISCHER, H. AND E. HAARER. Ztschr. f. physiol. Chem. **204**: 101, 1932.
(m) FISCHER, H. AND E. DUESBERG. Arch. f. exper. Path. u. Pharmakol. **166**: 95, 1932.
(n) See reference 46b.
(o) FISCHER, H. AND W. GLEIM. J. v. Liebig's Ann. d. Chem. **521**: 157, 1935.
(p) FISCHER, H. AND H. LIBOWITZKY. Ztschr. f. physiol. Chem. **241**: 220, 1936.
(q) FISCHER, H. AND C. v. SEEMANN. Ztschr. f. physiol. Chem. **242**: 133, 1936.
(r) FISCHER, H. AND H. J. HOFMANN. Ztschr. f. physiol. Chem. **246**: 15, 1937.
(s) FISCHER, H. AND A. MÜLLER. Ztschr. f. physiol. Chem. **246**: 43, 1937.
(36a) FOURIE, P. J. J. Onderstepoort J. Vet. Sc. **7**: 535, 1936.
(b) FOURIE, P. J. J. AND C. R. RIMINGTON. Onderstepoort J. Vet. Sc. **10**: 431, 1938.
(37) FRAENKEL, E. Virchow's Arch. **248**: 125, 1924.
(38a) FRANKE, K. Ztschr. f. klin. Med. **130**: 222, 1936.
(b) FRANKE, K. AND R. FIKENTSCHER. München. med. Wchnschr. **82**: 171, 1935.
(c) FRANKE, K. AND S. LITZNER. Ztschr. f. klin. Med. **129**: 115, 1935.
(39) FISCHEL, W. AND J. HÜHNERFELD. Ztschr. f. d. ges. exper. Med. **101**: 425, 1937.
(40) GAFFRON, H. Biochem. Ztschr. **179**: 157, 1926.
(41a) GARROD, A. E. Inborn errors of metabolism. H. Frowde, London, 1923. 2. ed.
(b) GARROD, A. E. J. Physiol. **13**: 598, 1892.
(c) GARROD, A. E. Quart. J. Med. **29**: 473, 1936.
(42) GEISSLER, J. Klin. Wchnschr. **18**: 378, 1939.
(43) GERMUTH, F. G. Am. J. Pharm. **99**: 685, 1927.
(44) GILDEMEISTER, H. Ztschr. f. d. ges. exper. Med. **102**: 58, 1937.
(45) GOECKERMAN, H. W., A. E. OSTERBERG AND C. SHEARD. Arch. Dermat. and Syph. **20**: 501, 1929.

(46a) GOTTRON, H. AND F. ELLINGER. Arch. f. Dermat. u. Syph. **164:** 11, 1931.
(b) GOTTRON, H. AND F. ELLINGER. Arch. f. Dermat. u. Syph. **167:** 325, 1933.
(47) GRAY, A. M. H. Quart. J. Med. **19:** 381, 1926.
(48a) GROTEPASS, W. Ztschr. f. physiol. Chem. **205:** 193, 1932.
(b) GROTEPASS, W. AND Z. HULST. Nederl. tijdschr. v. geneesk. **79:** 1780, 1935.
(c) GROTEPASS, W. Nederl. tijdschr. v. geneesk. **81:** 362, 1937.
(d) GROTEPASS, W. Ztschr. f. physiol. Chem. **253:** 276, 1938.
(e) GROTEPASS, W. AND A. DEFALQUE. Ztschr. f. physiol. Chem. **252:** 155, 1938.
(49) GRÜNEWALD, E. A. J. f. Psychol. u. Neurol. **29:** 403, 1923.
(50a) GÜNTHER, H. Ergebn. d. allg. Path. u. path. Anat. **20:** pt. 1, 608, 1922.
(b) GÜNTHER, H. In: Handbuch der Krankheiten des Blutes und der blutbildenden Organe (Schittenhelm) **2:** 622, 1925.
(51) HÄCKER, W. Arch. f. exper. Path. u. Pharmakol. **184:** 723, 1937.
(52) HALTER, K. Arch. f. Dermat. u. Syph. **178:** 340, 1938.
(53) HAMMARSTEN, O. Skandinav. Arch. f. Physiol. **3:** 319, 1892.
(54) HAMMER, H. Virchow's Arch. **277:** 159, 1930.
(55) HARBITZ, F. Arch. Int. Med. **33:** 632, 1924.
(56a) HAUROWITZ, F. Ztschr. f. physiol. Chem. **188:** 161, 1930.
(b) HAUROWITZ, F. Arch. f. Verdauungskr. **50:** 33, 1931.
(57a) HAUSMANN, W. AND H. HAXTHAUSEN. Sonderbände z. Strahlentherapie **11:** 1929.
(b) HAUSMANN, W. AND F. M. KUEN. Biochem. Ztschr. **265:** 105, 1933.
(58) HAXTHAUSEN, H. Dermat. Wchnschr. **84:** 827, 1927.
(59a) HEGLER, C., E. FRAENKEL AND O. SCHUMM. Deutsch. med. Wchnschr. **39:** 842, 1913.
(b) HEGLER, C., E. FRAENKEL AND O. SCHUMM. Deutsch. med. Wchnschr. **42:** 1242, 1916.
(60a) HEROLD, L. Arch. f. Gynäk. **158:** 213, 1934.
(b) HEROLD, L. Arch. f. Gynäk. **159:** 35, 1935.
(61a) HIJMANS VAN DEN BERGH, A. A., REGNIERS AND P. MÜLLER. Arch. f. Verdauungskr. **42:** 302, 1928.
(b) HIJMANS VAN DEN BERGH, A. A., W. GROTEPASS AND F. E. REVERS. Klin. Wchnschr. **11:** 1534, 1932.
(c) HIJMANS VAN DEN BERGH, A. A. AND W. GROTEPASS. Klin. Wchnschr. **12:** 586, 1933.
(d) HIJMANS VAN DEN BERGH, A. A. AND W. GROTEPASS. Compt. rend. Soc. de biol. **121:** 1253, 1936.
(62a) HINSBERG, K. Klin. Wchnschr. **18:** 180, 1939.
(b) HINSBERG, K. AND R. AMMON. Ztschr. f. physiol. Chem. **246:** 139, 1937.
(c) HINSBERG, K. AND R. MERTEN. Biochem. Ztschr. **299:** 137, 1938.
(d) HINSBERG, K. AND W. RODENWALD. Arch. f. exper. Path. u. Pharmakol. **191:** 1, 1939.
(63) HOAGLAND, R. J. Agric. Res. **7:** 41, 1916.
(64a) HOERBURGER, W. Inaugural Dissertation, Erlangen, 1933.
(b) HOERBURGER, W. AND H. FINK. Ztschr. f. physiol. Chem. **236:** 136, 1935.
(c) HOERBURGER, W. AND W. SCHULZE. Arch. f. Dermat. u. Syph. **175:** 671, 1937.

(65) HOESCH, K. AND C. CARRIÉ. Ztschr. f. klin. Med. **129**: 214, 1935.
(66a) HOLDEN, H. F. Australian J. Exper. Biol. and M. Sc. **15**: 409, 1937.
(b) HOLDEN, H. F. Australian J. Exper. Biol. and M. Sc. **16**: 153, 1938.
(67) HÜBNER, K. H. Arch. f. Dermat. u. Syph. **174**: 38, 1936.
(68) HÜHNERFELD, J. Ztschr. f. d. ges. Neurol. u. Psychiat. **154**: 799, 1936
(69) HUTSCHENREUTER, R. Ztschr. f. physiol. Chem. **222**: 161, 1933.
(70a) JAKOB, A. Arch. f. exper. Path. u. Pharmakol. **189**: 272, 1938.
(b) JAKOB, A. Klin. Wchnschr. **18**: 507, 1939.
(71) JOST, H. Klin. Wchnschr. **6**: 827, 1927.
(72a) KÄMMERER, H. Deutsch. Arch. f. klin. Med. **145**: 257, 1924.
(b) KÄMMERER, H. AND H. WEISBECKER. Arch. f. exper. Path. u. Pharmakol. **111**: 263, 1926.
(c) KÄMMERER, H. AND J. GÜRSCHING. Verhandl. d. deutsch. Gesellsch. f. inn. Med. **41**: 486, 1929.
(d) KÄMMERER, H. Verhandl. d. deutsch. Gesellsch. f. inn. Med. **45**: 28, 1933.
(73a) KAPP, E. M. Brit. J. Exper. Path. **20**: 33, 1939.
(b) KAPP, E. M. AND A. F. COBURN. Brit. J. Exper. Path. **17**: 255, 1936; and personal communication.
(74) KAUNITZ, H. Ztschr. f. klin. Med. **133**: 552, 1938.
(75) KEYS, A. AND J. T. BRUGSCH. J. Am. Chem. Soc. **60**: 2135, 1938.
(76) KIRSTAHLER, A. Tabulae biologicae **7**: 48, 1931.
(77) KÖNIGSDÖRFFER, H. Strahlentherapie **28**: 132, 1928.
(78) KURT, W. Schweiz. med. Wchnschr. **69**: 73, 1939.
(79a) LAGEDER, K. Arch. f. Verdauungskr. **56**: 237, 1934.
(b) LAGEDER, K. Klin. Wchnschr. **15**: 296, 1936.
(80) LANGECKER, H. Ztschr. f. d. ges. exper. Med. **68**: 258, 1929.
(81a) DE LANGEN, C. D. AND W. GROTEPASS. Acta med. Scandinav. **94**: 245, 1938.
(b) DE LANGEN, C. D. AND W. GROTEPASS. Acta med. Scandinav. **97**: 29, 1938.
(82) LAUBENDER, W. AND K. MONDEN. Arch. f. exper. Path. u. Pharmakol. **188**: 562, 1938.
(83a) LEMBERG, R. Biochem. J. **29**: 1322, 1935.
(b) LEMBERG, R. Ann. Rev. Biochemistry **7**: 421, 1938.
(c) LEMBERG, R., B. CORTIS-JONES AND M. NORRIE. Biochem. J. **32**: 149, 171, 1938.
(84) LIBOWITZKY, H. AND K. F. SCHEID. Klin. Wchnschr. **17**: 156, 1938.
(85) LICHTWITZ, L. Pathologie der Funktionen und Regulationen. A. W. Sijthoff, Leiden, 1936.
(86) LIEBIG, H. Arch. d. exper. Path. u. Pharmakol. **125**: 16, 1927.
(87) LIGNAC, G. O. E. Krankheitsforsch. **1**: 177, 1925.
(88) LOCALIO, S. A., S. M. SCHWARTZ AND C. GANNON. Personal communication.
(89) LÖFFLER, W. Biochem. Ztschr. **98**: 105, 1919.
(90) LORENTE, L. AND H. SCHOLDERER. Arch. f. Verdauungskr. **59**: 188, 1936.
(91) LUDY, J. B. AND E. F. CORSON. Arch. Dermat. and Syph. **37**: 403, 1938.
(92) MAASE, C. Ztschr. f. klin. Med. **99**: 270, 1924.

(93) MACKEY, L. AND A. E. GARROD. Quart. J. Med. **19:** 357, 1926.
(94) v. MALLINCKRODT-HAUPT, A. S. Klin. Wchnschr. **18:** 153, 1939.
(95) MAGENDANTZ, H. Nervenarzt **9:** 76, 1936.
(96) MARIQUE, P. Bull. Soc. de. chim. biol. **20:** 325, 1938.
(97) MARQUARDT. Zentralbl. f. Haut- u. Geschlechtskr. **53:** 525, 1936.
(98) MASON, V. R., C. COURVILLE AND E. ZISKIND. Medicine **12:** 355, 1933.
(99) MASSA, M. Riforma med. **48:** 1669, 1932.
(100) MATHEWS, F. P. Arch. Path. **23:** 399, 1937.
(101) MANCKE, R. Deutsch. Arch. f. klin. Med. **170:** 358, 1931.
(102) MAYER, R. M. Ztschr. f. physiol. Chem. **179:** 99, 1928.
(103) MCMASTER, P. D., J. O. BROUN AND P. ROUS. J. Exper. Med. **37:** 395, 421, 733, 1923.
(104) MCFARLAND, A. R. AND W. H. STRAIN. Arch. Dermat. and Syph. **38:** 727, 1938.
(105a) MERTENS, E. Klin. Wchnschr. **16:** 61, 1936.
(b) MERTENS, E. Ztschr. f. physiol. Chem. **238:** 1, 1936.
(c) MERTENS, E. Ztschr. f. physiol. Chem. **250:** 57, 1937.
(d) MERTENS, E. Ztschr. f. physiol. Chem. **258:** supp. 1, 1939.
(106) MEYER-BETZ, F. Deutsch. Arch. f. klin. Med. **112:** 476, 1913.
(107) MICHELI, F. AND G. DOMINICI. Deutsch. Arch. f. klin. Med. **171:** 154, 1931.
(108) MICHELI, F. AND G. DOMINICI. Minerva med. **2:** 469, 505, 1930.
(109) MINNIBECK, H. AND U. STRASSER. Klin. Wchnschr. **17:** 989, 1938.
(110) NENCKI, M. AND N. SIEBER. Arch. f. exper. Path. u. Pharmakol. **18:** 401, 1884.
(111) NENCKI, M. AND N. SIEBER. Arch. f. exper. Path. u. Pharmakol. **24:** 430, 1888.
(112) NEUBAUER, O. Arch. f. exper. Path. u. Pharmakol. **43:** 456, 1900.
(113) PEACHEY, C. H., K. DOBRINER AND W. H. STRAIN. New York State J. Med. **38:** 848, 1938.
(114) PERUTZ, A. Arch. Dermat. u. Syph. **124:** 531, 1917.
(115) POLSON, C. Brit. J. Exper. Path. **8:** 197, 1927.
(116) RASK, E. N. AND W. H. HOWELL. Am. J. Physiol. **84:** 363, 1928.
(117) REITLINGER, K. AND P. KLEE. Arch. f. exper. Path. u. Pharmakol. **127:** 277, 1928.
(118a) RIMINGTON, C. Onderstepoort J. Vet. Sc. **7:** 567, 1936.
(b) RIMINGTON, C. Biochem. J. **32:** 460, 1938.
(c) RIMINGTON, C. Compt. rend. Trav. Lab. Carlsberg, Ser. chim. **22:** 454, 1938.
(d) RIMINGTON, C. Ztschr. f. physiol. Chem. **259:** 45, 1939.
(e) RIMINGTON, C. Proc. Roy. Soc. London (Ser. B) **127:** 106, 1939.
(f) RIMINGTON, C. Proc. Roy. Soc. Med. **32:** 351, 1939.
(g) RIMINGTON, C. AND A. W. HEMMINGS. Lancet **1:** 770, 1938.
(h) RIMINGTON, C. AND A. W. HEMMINGS. Biochem. J. **33:** 960, 1939.
(i) RIMINGTON, C., G. C. S. ROETS AND P. J. J. FOURIE. Onderstepoort J. Vet. Sc. **10:** 421, 1938.
(119) ROETS, G. C. S. Onderstepoort J. Vet. Sc. **11:** 385, 1938.
(120) RONCORONI, C. Pathologica **27:** 737, 1935.

(121a) Roth, E. Ztschr. f. klin. Med. **129:** 123, 1936.
(b) Roth, E. Deutsch. Arch. f. klin. Med. **178:** 185, 1935.
(122) Rothemund, P. J. Am. Chem. Soc. **58:** 625, 1936.
(123) Sachs, P. Klin. Wchnschr. **10:** 1123, 1931.
(124) Saillet. Rev. de méd. **16:** 542, 1896.
(125) Salkowski, E. Ztschr. f. physiol. Chem. **15:** 286, 1891.
(126) Schmey, H. Frankfurt. Ztschr. f. Path. **12:** 218, 1913.
(127) Schönheimer, R. Ztschr. f. physiol. Chem. **180:** 144, 1929.
(128) Schønheyder, F. J. Biol. Chem. **123:** 491, 1938.
(129a) Schreus, H. T. Klin. Wchnschr. **14:** 1717, 1935.
(b) Schreus, H. T. and C. Carrié. Strahlentherapie **40:** 340, 1931.
(c) Schreus, H. T. and C. Carrié. Ztschr. f. klin. Med. **125:** 330, 1933.
(130a) Schumm, O. Die spektrochemische Analyse natürlicher organischer Farbstoffe. G. Fischer, Jena, 1927.
(b) Schumm, O. Ztschr. f. physiol. Chem. **105:** 158, 1919.
(c) Schumm, O. Ztschr. f. physiol. Chem. **126:** 169, 1923.
(d) Schumm, O. Ztschr. f. physiol. Chem. **153:** 225, 1926.
(e) Schumm, O. Ztschr. f. physiol. Chem. **158:** 77, 1926.
(f) Schumm, O. Ztschr. f. physiol. Chem. **169:** 52, 1927.
(g) Schumm, O. Arch. f. exper. Path. u. Pharmakol. **191:** 529, 1939.
(131a) Scolari, E. Gior. ital. di dermat. e sif. **76:** 1183, 1935.
(b) Scolari, E. Gior. ital. di dermat. e sif. **78:** 5, 1937.
(132) Seggel, K. A. Folia haematol. **54:** 374, 1936.
(133) Shibuya, H. Strahlentherapie **17:** 412, 1924.
(134) Silver, B. and M. Elliott. J. A. M. A. **112:** 723, 1939.
(135a) Smetana, H. J. Exper. Med. **47:** 593, 1928.
(b) Smetana, H. J. Biol. Chem. **124:** 667, 1938.
(c) Smetana, H. J. Biol. Chem. **125:** 741, 1938.
(136) Snapper, I. and S. van Creveld. Ergebn. d. inn. Med. u. Kinderh. **32:** 1, 1927.
(137) Spies, T. D., Y. Sasaki and E. S. Gross. Southern M. J. **31:** 483, 1938.
(138) Stern, K. G. J. Biol. Chem. **112:** 661, 1936.
(139) Stockvis, B. J. Ztschr. f. klin. Med. **28:** 1, 1895.
(140) Strauch, C. B. Am. J. Dis. Child. **40:** 800, 1930.
(141) Pi Suñer, A. J. de physiol. et path. gén. **5:** 1052, 1903.
(142) Sumner, J. B. and S. F. Howell. Enzymologia **1:** 133, 1936.
(143) Supniewski, J. V. J. Physiol. **64:** 30, 1927.
(144) Taylor, A. E. Centralbl. f. inn. Med. **18:** 873, 1897.
(145a) Thiel, W. Verhandl. d. deutsch. Gesellsch. inn. Med. **45:** 81, 1933.
(b) Thiel, W. Klin. Wchnschr. **13:** 700, 1934.
(146a) Thomas, J. and E. J. Bigwood. Compt. rend. Soc. de biol. **118:** 381, 1935.
(b) Thomas, J. Bull. Soc. de chim. biol. **20:** 471, 635, 878, 1058, 1300, 1938.
(147a) Tropp, C. and K. Siegler. Deutsch. Arch. f. klin. Med. **180:** 402, 1937.
(b) Tropp, C. and L. Penew. Deutsch. Arch. f. klin. Med. **180:** 411, 1937.
(c) Tropp, C. Arch. f. Dermat. u. Syph. **177:** 112, 1938.
(148a) Turner, W. J. J. Biol. Chem. **118:** 519, 1937.
(b) Turner, W. J. Arch. Int. Med. **61:** 762, 1938.

(c) TURNER, W. J. AND M. E. OBERMAYER. Arch. Dermat. and Syph. **37:** 549, 1938.
(149) URBACH, E. AND J. BLÖCH. Wien. klin. Wchnschr. **47:** 527, 1934.
(150) VAHLQUIST, B. Ztschr. f. physiol. Chem. **259:** 213, 1939.
(151) VAN LEERSUM, E. C. J. Biol. Chem. **58:** 835, 1924.
(152a) VANNOTTI, A. Ergebn. d. inn. Med. u. Kinderh. **49:** 337, 1935.
(b) VANNOTTI, A. Porphyrine und Porphyrinkrankheiten. J. Springer, Berlin, 1937.
(c) VANNOTTI, A. AND E. NEUHAUS. Ztschr. f. d. ges. exper. Med. **97:** 398, 1935.
(d) VANNOTTI, A. Ztschr. f. d. ges. exper. Med. **97:** 377, 1935.
(e) VANNOTTI, A. Helvet. med. acta **3:** 633, 1936.
(153a) VIGLIANI, E. C. AND P. SASSO. Raasegna di med. appl. al lavoro indust. **5:** 305, 1934.
(b) VIGLIANI, E. C. AND C. ANGELERI. Klin. Wchnschr. **15:** 700, 1936.
(c) VIGLIANI, E. C. AND J. WALDENSTRÖM. Deutsch. Arch. f. klin. Med. **180:** 182, 1937.
(d) VIGLIANI, E. C. AND H. LIBOWITZKY. Klin. Wchnschr. **16:** 1243, 1937.
(e) VIGLIANI, E. C. AND B. SONZINI. Arch. per le sc. med. **65:** 363, 1938.
(f) VIGLIANI, E. C. Arch. per le sc. med. **65:** 391, 1938.
(g) VIGLIANI, E. C., C. ANGELERI AND M. SANÒ. Arch. per le sc. med. **65:** 423, 1938.
(154a) VÖLKER, O. J. f. Ornithol. **86:** 436, 1938.
(b) VÖLKER, O. Ztschr. f. physiol. Chem. **258:** 1, 1939.
(155a) WALDENSTRÖM, J. Studien über Porphyrie. Acta med. Scandinav. 1937, Supp. 82.
(b) WALDENSTRÖM, J. Deutsch. Arch. f. klin. Med. **178:** 38, 1935.
(c) WALDENSTRÖM, J. Ztschr. f. physiol. Chem. **239:** iii, 1936.
(d) WALDENSTRÖM, J. Acta med. Scand. **83:** 281, 1934.
(e) WALDENSTRÖM, J., H. FINK AND W. HOERBURGER. Ztschr. f. physiol. Chem. **233:** 1, 1935.
(f) WALDENSTRÖM, J. AND B. VAHLQUIST. Ztschr. f. physiol. Chem. **260:** 189, 1939.
(g) WALDENSTRÖM, J. AND S. WENDT. Ztschr. f. physiol. Chem. **259:** 157, 1939.
(156) WATSON, C. J. In: Handbook of hematology (Downey) **4:** 2445, 1938.
(a) WATSON, C. J. Ztschr. f. physiol. Chem. **204:** 57, 1932.
(b) WATSON, C. J. J. Clin. Investigation **14:** 106, 1935.
(c) WATSON, C. J. J. Clin. Investigation **14:** 110, 1935.
(d) WATSON, C. J. J. Clin. Investigation **14:** 116, 1935.
(e) WATSON, C. J. J. Clin. Investigation **15:** 327, 1936.
(f) WATSON, C. J. J. Clin. Investigation **16:** 383, 1937.
(g) WATSON, C. J. Proc. Soc. Exper. Biol. and Med. **39:** 514, 1938.
(h) WATSON, C. J. AND W. O. CLARKE. Proc. Soc. Exper. Biol. and Med. **36:** 65, 1937.
(i) WATSON, C. J. In: Oxford medicine (Christian) **4:** 228 (1–34), 1938.
(j) WATSON, C. J. In: A symposium on the blood and blood-forming organs. Univ. of Wisconsin Press, 1939, p. 14.

(157) WENDLBERGER, J. AND A. KLEIN. Arch. f. Dermat. u. Syph. **176**: 522, 1938.
(158) WEISS, H. Deutsch. Arch. f. klin. Med. **149**: 255, 1925.
(159) WIEN, R. Quart. J. Pharm. **11**: 217, 1938.
(160) WOHLGEMUTH, J. AND E. SZÖRÉNYI. Biochem. Ztschr. **264**: 389, 1933.
(161a) ZEILE, K. AND B. RAU. Ztschr. f. physiol. Chem. **250**: 197, 1937.
(b) ZEILE, K. AND F. REUTER. Ztschr. f. physiol. Chem. **221**: 101, 1933.
(162) ZOON, J. J. Strahlentherapie **61**: 640, 1938.
(163) ZORN, O. Klin. Wchnschr. **17**: 1576, 1938.